Assessment of genetically modified oilseed rape MON 94100 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-169)

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Assessment of genetically modified oilseed rape MON 94100 for food and feed uses, under regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2020-169)

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Abstract

Oilseed rape MON 94100 was developed to confer tolerance to dicamba herbicide. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between oilseed rape MON 94100 and its conventional counterpart needs further assessment, except for the levels of carbohydrates, calcium and ADF in seeds, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the dicamba mono-oxygenase (DMO) protein as expressed in oilseed rape MON 94100. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of oilseed rape MON 94100. In the context of this application, the consumption of food and feed from oilseed rape MON 94100 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that oilseed rape MON 94100 is as safe as the conventional counterpart and non-GM oilseed rape reference varieties tested, and no post-market monitoring of food/feed is considered necessary. In the case of accidental release of viable oilseed rape MON 94100 seeds into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan and reporting intervals are in line with the intended uses of oilseed rape MON 94100. The GMO Panel concludes that oilseed rape MON 94100 is as safe as its conventional counterpart and the tested non-GM oilseed rape reference varieties with respect to potential effects on human and animal health and the environment.

Keywords: GM, genetic engineering, oilseed rape (*Brassica napus*), MON 94100, DMO, import and processing

Requestor: Competent Authority of The Netherlands

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Summary

Following the submission of application EFSA-GMO-NL-2020-169 under Regulation (EC) No 1829/2003 from Bayer Agriculture BV (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide tolerant oilseed rape MON 94100 according to Regulation (EU) No 503/2013. The scope of application EFSA-GMO-NL-2020-169 is for import, processing and food and feed uses within the European Union (EU) of oilseed rape MON 94100 and does not include cultivation in the EU.

In this scientific opinion, the GMO Panel reports on the outcome of its risk assessment of oilseed rape MON 94100 according to the scope of the application EFSA-GMO-NL-2020-169. The GMO Panel conducted the assessment of oilseed rape MON 94100 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of GM plants. The molecular characterisation data establish that oilseed rape MON 94100 contains a single insert consisting of one copy of the *dmo* expression cassette. The quality of the sequencing methodology and data sets was assessed by the GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note. Updated bioinformatic analyses of the sequences encoding the newly expressed protein and open reading frames (ORFs) present within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the dicamba mono-oxygenase (DMO) protein (consisting of two variants, DMO and DMO + 27) is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of the oilseed rape MON 94100-produced DMO and DMO + 27 indicate that they are equivalent to their soybean counterparts that were previously assessed (EFSA GMO Panel, 2013).

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between oilseed rape MON 94100 and its conventional counterpart needed further assessment, except for the levels of carbohydrates, calcium and ADF in seeds, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO protein as expressed in oilseed rape MON 94100. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of oilseed rape MON 94100. In the context of this application, the consumption of food and feed from oilseed rape MON 94100 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that oilseed rape MON 94100 is as safe as the conventional counterpart and non-GM oilseed rape reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced trait, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, oilseed rape MON 94100 would not raise safety concerns in the case of accidental release of viable GM oilseed rape seeds into the environment. The post-market environmental monitoring (PMEM) plan and reporting intervals are in line with the intended uses of oilseed rape MON 94100.

The GMO Panel considered the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant peer-reviewed publications on oilseed rape MON 94100. The GMO Panel concludes that oilseed rape MON 94100 is as safe as its conventional counterpart and the tested non-GM oilseed rape reference varieties with respect to potential effects on human and animal health and the environment.

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1. Introduction

The scope of the application EFSA-GMO-NL-2020-169 is for food and feed uses, import and processing of oilseed rape MON 94100 and does not include cultivation in the European Union (EU).

1.1. Background

On 16 November 2020, the European Food Safety Authority (EFSA) received from the Competent Authority of The Netherlands application EFSA-GMO-NL-2020-169 for authorisation of oilseed rape (*Brassica napus*) MON 94100 (Unique Identifier MON-941ØØ-2), submitted by Bayer Agriculture BV (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003[1]. Following receipt of application EFSA-GMO-NL-2020-169, EFSA informed EU Member States and the European Commission, and made the application available to them. Simultaneously, EFSA published a summary of the application.[2] EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EC) No 503/2013[3], with EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 25 March 2021, EFSA declared the application valid. From 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-NL-2020-169. Such time limit was extended whenever EFSA and/or the 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). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of the EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC[4]. The EU Member States had 3 months to make their opinion known on application EFSA-GMO-NL-2020-169 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 MON 94100 in the context of its scope as defined in application EFSA-GMO-NL-2020-169. 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 and thus will be part of the EFSA overall opinion in accordance with Articles 6(5) and 18(5). In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[5]

2. Data and methodologies

2.1. Data

The GMO Panel based its scientific risk assessment of oilseed rape MON 94100 on the valid application EFSA-GMO-NL-2020-169, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications.

2.2. Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, its applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a,b, 2015a, 2017, EFSA

[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/study-inventory/EFSA-Q-2020-00749
[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] 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.
[5] These particulars are available online: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2020-00749

Scientific Committee, 2011), explanatory notes and statements (i.e. EFSA GMO Panel, 2010b, 2018; EFSA, 2010, 2014, 2017, 2018, 2019a,b) for the risk assessment of GM plants.

For this application, in the context of the contracts [OC/EFSA/GMO/2018/04], [OC/EFSA/GMO/2018/02], [EOI/EFSA/SCIENCE/2020/01 – CT01GMO and CT02GMO] and [OC/EFSA/GMO/2020/01], the contractors performed preparatory work for the evaluation of the applicant's literature search, of the methods applied for the statistical analysis and statistical analysis of the 90-day toxicity study and of the completeness and quality of DNA sequencing information.

3. Assessment

3.1. Introduction

Oilseed rape MON 94100 was developed to confer tolerance to dicamba herbicide. Oilseed rape MON 94100 expresses two variants of the DMO protein: DMO and DMO + 27. The DMO protein demethylates dicamba, producing 3,6-dichlorosalicylic acid and formaldehyde, conferring tolerance to dicamba-based herbicides. It should be noted that the assessment of herbicide residues relevant for this application is in the remit of the EFSA Plant Health and Pesticides Residues Unit.

3.2. Systematic literature review[6]

The GMO Panel assessed the applicant's literature searches on oilseed rape MON 94100, which include a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application EFSA-GMO-NL-2020-169. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value of undertaking a systematic review for oilseed rape MON 94100 at present. The GMO Panel considered the overall quality of the performed literature searches acceptable.

The literature searches did not identify any relevant peer-reviewed publications on oilseed rape MON 94100. However, the applicant retrieved three relevant records of assessment issued by Canada (Health Canada), Australia and New Zealand (FSANZ) and Japan (MAFF) based on MON 94100.

None of the relevant records identified through the literature searches reported information pointing to safety issues associated with MON 94100 relevant to the scope of this application.

3.3. Molecular characterisation[7]

3.3.1. Transformation process and vector constructs

Oilseed rape MON 94100 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Hypocotyl segments of oilseed rape variety 65037 were co-cultured with a disarmed *A. tumefaciens* strain AB33 containing the vector PV-BNHT508701. The plasmid PV-BNHT508701 used for the transformation contains two separate T-DNAs, each with a right and left border. T-DNA I carries one expression cassette containing the following genetic elements:

- The *dmo* expression cassette consisting of the PClSV promoter from peanut chlorotic streak caulimovirus, the 5′ untranslated leader sequence from the RNA of tobacco etch virus (TEV), a sequence encoding 27 amino acids of the chloroplast targeting peptide from the Rubisco (*rbcS)* gene of *Pisum sativum*, and from an intervening sequence (DMO + 27), the coding sequence of the dicamba mono-oxygenase gene (*dmo*) from *Stenotrophomonas maltophilia*, and the 3′ untranslated sequence of the *guf-Mt1* gene from *Medicago truncatula*.

T-DNA II carries two expression cassettes containing the following genetic elements:

- The *aadA* expression cassette consisting of the enhancer from the 35S RNA of figwort mosaic virus (FMV), the promoter, leader and intron sequences of the EF–1α gene from *Arabidopsis thaliana*, the targeting sequence of the *ShkG* gene from *Arabidopsis thaliana*, the coding sequence of the *aadA* gene, the 3′ untranslated sequence of the E9 gene from *Pisum sativum*.

[6] Dossier: Part II – Section 7; additional information provided: 19/10/2021, 13/5/2022, 24/5/2022.
[7] Dossier: Part II – Section 1.2; additional information provided: 09/06/2021, 20/8/2021, 19/10/2021, 19/1/2022, 13/5/2022.

– The *splA* expression cassette consisting of 5′ UTR leader, promoter and enhancer sequence of an unknown seed protein gene (*Usp*) from *Vicia faba*, the coding sequence of the *splA* gene from *Agrobacterium tumefaciens* and the 3′ untranslated sequence of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens*.

The vector backbone contains elements necessary for the maintenance and selection of the plasmid in bacteria.

T-DNA II was used for selecting the transformed plants and after self-pollination of R0 plants, only those in which TDNA II was segregated away were selected in the R1 generation.

3.3.2. Transgene constructs in the GM plant

Molecular characterisation of oilseed rape MON 94100 was performed by next-generation sequencing (NGS) and junction sequence analysis (JSA) in order to determine insert copy number and to confirm the absence of plasmid backbone and T-DNA II sequences, and NGS sequencing on PCR amplified fragments to determine size and organisation of the inserted sequences. The approach used is acceptable in terms of coverage and sensitivity. Overall, the quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note (2018).

NGS/JSA of the whole genome indicated that oilseed rape MON 94100 contains a single insert, consisting of a single copy of the T-DNA I in the same configuration as in the PV-BNHT508701 transformation vector. NGS/JSA also confirmed the absence of plasmid backbone and T-DNA II sequences in the oilseed rape genome.

The nucleotide sequence of the entire insert of oilseed rape MON 94100 together with 1,000 bp of the 5′ and 1,000 bp of the 3′ flanking regions was determined. The insert of 2,913 bp is identical to the T-DNA I of PV-BNHT508701, with the exception of 216 bp deleted from the T-DNA RB and 161 bp deleted from the T-DNA LB.

A comparison with the pre-insertion locus indicated that 8 bp were deleted from the oilseed rape genomic DNA. The possible interruption of known endogenous oilseed rape genes by the insertion in oilseed rape MON 94100 was evaluated by bioinformatic analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The results of these analyses do not indicate the interruption of any known endogenous gene in oilseed rape MON 94100.

The results of segregation (see Section 3.3.5) and bioinformatic analyses are compatible with a single insertion in the nuclear genome.

Two variants of the DMO protein are present in MON 94100, DMO and DMO + 27 previously assessed in EFSA GMO Panel 2013 (EFSA-GMO-NL-2011-93). Updated bioinformatic analyses of the amino acid sequence of the DMO + 27 protein (which contains the DMO amino acid sequence) reveal no significant similarities to toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA also do not indicate significant similarities to toxins and allergens.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis for oilseed rape MON 94100 to microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.6.1.2.

3.3.3. Protein characterisation and equivalence

Oilseed rape MON 94100 expresses a new protein: DMO, a dicamba mono-oxygenase conferring resistance to dicamba herbicide. A set of biochemical methods was employed to demonstrate the equivalence between the oilseed rape MON 94100 DMO protein and the soybean MON 87708 DMO protein assessed and considered safe by the EFSA GMO panel (EFSA GMO Panel, 2013). Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

DMO protein characterisation and equivalence

The DMO precursor protein undergoes alternative processing, resulting in two monomeric forms (DMO and DMO + 27) previously assessed in EFSA GMO Panel 2013 (EFSA-GMO-NL-2011-93). The characterisation of the DMO and DMO + 27 protein variants was conducted in parallel to the soybean

MON 87708-derived DMO protein variants that have already been assessed for safety in the context of its intended use (EFSA GMO Panel, 2013).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis showed that oilseed rape MON 94100 and soybean MON 87708 DMO proteins migrated at the same position. The expected molecular weights of DMO + 27 and DMO were calculated to be ~ 39.4 and 38.0 kDa, respectively, and were comparably immunoreactive to DMO protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the DMO proteins were glycosylated. Amino acid sequence analysis of the plant-derived DMO proteins by mass spectrometry (MS) methods showed that the DMO proteins matched the deduced sequence as defined by the *dmo* gene, for DMO and DMO + 27 present in oilseed rape MON 94100. These sequence analysis data were consistent with the previously analysed MON 87708-derived DMO protein variants. In addition, the MS data showed that the DMO N-terminal methionine was removed. Such modifications are common in eukaryotic proteins (e.g. Polevoda and Sherman, 2000). N-terminal sequence analysis of the DMO purified from oilseed rape MON 94100 confirmed that, as expected, MON 94100 produced two variants of the DMO protein, DMO + 27 and DMO. Functional equivalence was demonstrated in comparison to the soybean MON 87708 by a biochemical in vitro activity assay which showed that oilseed rape and soybean-derived DMO proteins had comparable activity for the intended herbicide.

The protein characterisation data comparing the biochemical, structural and functional properties of oilseed rape and soybean-derived DMO proteins indicate that these proteins are equivalent.

3.3.4. Information on the expression of the insert

Protein levels of DMO (including DMO + 27) were analysed by enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across three locations in USA and two locations in Canada during the 2018 growing season. Samples analysed included seeds (BBCH99) from plants treated and not treated with dicamba. The mean values, standard errors and ranges of protein expression levels in seeds (n = 20) of the DMO protein used to estimate human and animal dietary exposure (see Section 3.5.5) are reported in Table 1.

Table 1: Mean values, standard errors and ranges of newly expressed protein in seeds [μg/g dry weight (dw) and μg/g fresh weight (fw)] from oilseed rape MON 94100 (n = 20)

	Dicamba treatment			
Tissues	**Not treated**		**Treated**	
	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Seed (BBCH99)				
DMO	0.63[(a)] ± 0.032[(b)] (0.36–0.87)[(c)]	0.59 ± 0.029 (0.33–0.81)	0.64 ± 0.068 (0.38–1.8)	0.59 ± 0.063 (0.35–1.6)

(a): Mean value.
(b): Standard error.
(c): Range.

3.3.5. Inheritance and stability of inserted DNA

Genetic stability of oilseed rape MON 94100 insert was assessed using NGS to sequence the insert and the flanking regions from five generations (R3, R3F1, R4, R5, R6) while segregation analysis was performed by PCR-based analysis from three consecutive generations (BC1F1, BC2F1 and BC3F1). The results indicate that all the plants tested retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations. The results support the presence of a single insertion, segregating in a Mendelian fashion.

3.3.6. Conclusion on molecular characterisation

The molecular characterisation data establish that oilseed rape MON 94100 contains a single insert consisting of one copy of the *dmo* expression cassette. Bioinformatic analyses of the sequences encoding the newly expressed protein and other ORFs within the insert or spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The

methodology used to quantify the levels of the DMO (including DMO + 27) protein is considered adequate. The protein characterisation data comparing the structural, biochemical and functional properties of the oilseed rape MON 94100-produced DMO and DMO + 27 indicate that they are equivalent to their soybean counterparts.

3.4. Comparative analysis[8]

3.4.1. Overview of studies conducted for the comparative analysis

Application EFSA-GMO-NL-2020-169 presents data on agronomic and phenotypic characteristics, as well as on seed composition of oilseed rape MON 94100 (Table 2).

Table 2: Overview of the comparative analysis studies to characterise the oilseed rape MON 94100 provided in application EFSA-GMO-NL-2020-169

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic, phenotypic and compositional	Field study, US and Canada, 2018, eight sites[(a)]	55076 × 65037	12[(b)]

GM: Genetically modified.

(a): Six field trials were located in United States at Power County, Idaho; Bonneville County, Idaho; Jerome County, Idaho; Grand Forks County, North Dakota; Brookings County, South Dakota and Grant County, Washington. Two field trials were located in Canada at Portage la Prairie R.M., Manitoba and Westlake-Gladstone R.M., Manitoba.

(b): Non-GM oilseed rape hybrid varieties with their corresponding maturity indicated in brackets were Advanta Hyola 575 CL (early), Brett Young 5535 CL (early), Brett Young 5545 CL (mid), Dekalb 71–30 CL (mid), Mycogen 2020 CL (mid), Mycogen 2022 CL (mid), Mycogen 2024 CL (mid), Pioneer 45H76 (mid), Pioneer 46H75 (mid), Rubisco Atomic TT (early), Rubisco DL1501 CL (mid-late) and Rubisco Trapper (early).

3.4.2. Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: oilseed rape MON 94100 not exposed to the intended herbicide, oilseed rape MON 94100 exposed to the intended herbicide, the comparator hybrid 55076 × 65037 and four non-GM reference varieties. The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b; 2011a). This includes, for each of the two treatments of oilseed rape MON 94100, the application of a difference test (between the GM oilseed rape and the comparator) and an equivalence test (between the GM oilseed rape and the set of non-GM commercial reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[9]

3.4.3. Suitability of selected test materials

3.4.3.1. Selection of the test materials

Oilseed rape variety 65037 was transformed to obtain oilseed rape MON 94100 which was then crossed with the variety 55076 to produce the hybrid used in the comparative analysis. In subsequent subsections, oilseed rape MON 94100 refers to hybrid (F_1 generation) obtained crossing GM oilseed rape line 65037 (carrying event MON 94100) with line 55076. The comparator used in the field trials is the non-GM oilseed rape hybrid 55076 × 65037, which has the same genetic background as MON 94100 (as documented by the pedigree) and is therefore considered to be the conventional counterpart.

Oilseed rape MON 94100 and the non-GM conventional counterpart, both of mid maturity, which is considered appropriate for growing in environments across the United States and Canada, where the comparative field trials were conducted.

Commercial non-GM reference varieties ranging from early to mid-late maturity were selected by the applicant and, at each selected site, three or four reference varieties were tested (see Table 2). On the basis of the provided information on maturity, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

[8] Dossier: Part II – Section 1.3; additional information provided: 09/06/2021, 19/1/2022.

[9] In detail, the four outcomes are: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

3.4.3.2. Seed production and quality

Seeds of oilseed rape MON 94100 and the conventional counterpart used in the 2018 field trials were harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event-specific PCR analysis.

The seeds were tested for their germination capacity under optimum and suboptimum temperature conditions.[10] Germination capacity of oilseed rape MON 94100 was compared with the one of its conventional counterparts and the results[11] of these studies indicate that the seed germination of oilseed rape MON 94100 was not different than that of its conventional counterpart.

3.4.3.3. Conclusion on suitability

The GMO Panel is of the opinion that oilseed rape MON 94100, the conventional counterpart and the non-GM oilseed rape reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

3.4.4. Representativeness of the receiving environments

3.4.4.1. Selection of field trial sites

The selected field trials sites were located in commercial oilseed rape-growing regions of the United States and Canada. The soil and climatic characteristics of the selected fields were diverse,[12] representing regions of diverse environmental conditions for oilseed rape cultivation. The GMO Panel considers that the selected sites reflect commercial oilseed rape-growing regions in which the test materials are likely to be grown.

3.4.4.2. Meteorological conditions

Maximum and minimum mean temperatures and sum of precipitations were provided on a weekly basis. No exceptional weather conditions were reported at any of the selected sites; therefore, the GMO Panel considers that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.

3.4.4.3. Management practices

The field trials included plots containing oilseed rape MON 94100, plots with the conventional counterpart and plots with non-GM oilseed rape reference varieties, managed according to local agricultural practices. In addition, the field trials included plots containing oilseed rape MON 94100 managed following the same agricultural practices, plus exposed to the intended dicamba containing herbicide. Dicamba containing herbicide was applied at the BBCH 14-16 growth stage. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the selected receiving environments.

3.4.4.4. Conclusion on representativeness

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and management practices of the field trial sites are typical for receiving environments where the tested materials could be grown.

3.4.5. Agronomic and phenotypic analysis

Data on 10 agronomic and phenotypic endpoints[13] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trials (see Table 2).

The statistical analysis (Section 3.4.2) was applied to all 10 endpoints, with the following results:

[10] Optimum temperature condition corresponds to an alternating temperature regime with 20°C was maintained ~ 16 h and 30°C for ~ 8 h. Suboptimum temperature conditions correspond to a constant temperature of ~ 5°C for 5 days followed by 25°C for 5 days.

[11] Oilseed rape MON 94100 and the non-GM comparator showed a mean germination of 100% under optimum and suboptimum temperature conditions.

[12] Soil types of the field trials were clay, silt loam, loam, silty clay loam, sandy loam and loamy sand; soil organic matter ranged from 0.7% to 2.8%; pH ranged from 5.7 to 8.1; historically, average temperatures and sum of precipitations during the usual crop growing season ranged, respectively, from 14.3 to 17.4°C and from 59 to 510 mm.

[13] Early stand count, days to flowering, plant height, lodging, final stand count, days to maturity, pod count, moisture, seed weight and yield.

- For oilseed rape MON 94100 (not treated with the intended herbicides), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, final stand count and moisture. All these endpoints fell under equivalence category I.
- For oilseed rape MON 94100 (treated with the intended herbicide), the test of difference identified statistically significant differences with the conventional counterpart for early stand count, final stand count, pod count and moisture. All these endpoints fell under equivalence category I.

3.4.6. Compositional analysis

Seeds of MON 94100 oilseed rape harvested from eight field trials (Table 3) were analysed for 56 constituents, including those recommended by OECD (OECD, 2011). The statistical analysis as described in Section 3.4.2 was not applied to 10 constituents[14] because their concentration in more than half of the samples was below the limit of quantification.

The statistical analysis was applied to a total of 46 constituents[15]; a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3:

- For MON 94100 oilseed rape not treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 13 endpoints. All these endpoints fell under equivalence category I or II except for carbohydrates which fell under equivalence category III, and acid detergent fibre (ADF) which fell under equivalence category IV.
- For MON 94100 oilseed rape treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 13 endpoints. All these endpoints fell under equivalence category I or II except for ADF and calcium which fell under equivalence category IV.

Table 3: Outcome of the comparative compositional analysis in seeds of oilseed rape MON 94100. The table shows the number of endpoints in each category

		Test of difference[(a)]			
		Not treated[(c)]		**Treated[(c)]**	
		Not different	**Significantly different**	**Not different**	**Significantly different**
Test of equivalence[(b)]	Category I/II	31	11[(d)]	32	11[(d)]
	Category III/IV	2[(e)]	2[(f)]	1[(e)]	2[(f)]
	Not categorised		–	–	–
	Total endpoints	46		46	

(a): Comparison between MON 94100 oilseed rape and the conventional counterpart.
(b): Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.
(c): Treated/not treated with the intended herbicide.
(d): Endpoints with significant differences between MON 94100 oilseed rape and the conventional counterpart and falling under equivalence category I-II. Not treated only: threonine, valine, palmitic acid (16:0), oleic acid (C18:1), linoleic acid (C18:2) and alkyl glucosinolates. Treated only: ash, moisture, protein, histidine, tryptophan and α-tocopherol. Both treated and not treated: NDF, glutamic acid, proline, sinapine and total glucosinolates.
(e): Endpoints with no significant differences between MON 94100 oilseed rape and the conventional counterpart and falling in equivalence categories III–IV. Not treated only: moisture and calcium. Treated only: carbohydrates.
(f): Endpoints with significant differences between MON 94100 oilseed rape and the conventional counterpart and falling in equivalence category III/IV. Not treated only: carbohydrates. Treated only: calcium. Both not treated and treated: ADF. Quantitative results for these endpoints are reported in Table 4.

[14] Caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), eicosadienoic acid (C20:2), erucic acid (C22:1) and docosadienoic acid (22:2).
[15] Proximates and fibre content (ash, carbohydrates, moisture, protein, total fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF)), minerals (calcium, phosphorus), vitamins (α-tocopherol and phylloquinone), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), fatty acids (palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0) and nervonic acid (C24:1)) and other compounds (total glucosinolates, alkyl glucosinolates, indolyl glucosinolates, phytic acid, sinapine and total tannins).

The GMO Panel assessed all the significant differences between MON 94100 oilseed rape and the conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between MON 94100 oilseed rape and the conventional counterpart and falling under equivalence category III/IV are given in Table 4.

Table 4: Quantitative results (estimated means and equivalence limits) for compositional endpoints in MON 94100 oilseed rape that are further assessed based on the results of the statistical analysis

Endpoint	MON 94100 oilseed rape		Conventional counterpart	Non-GM reference varieties	
	Not treated[(a)]	Treated[(a)]		Mean	Equivalence limits
Carbohydrates (% dw)	24.48*	24.68	24.92	26.79	24.85–28.73
ADF (% dw)	13.76*	13.82*	14.46	15.95	14.71–17.19
Calcium (% dw)	0.35	0.34*	0.36	0.43	0.37–0.49

dw: dry weight.
(a): Treated with the intended herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid).
For MON 94100 oilseed rape, significantly different values are marked with an asterisk, while the outcomes of the test of equivalence are differentiated by greyscale backgrounds: light grey (equivalence category III) and dark grey (equivalence category IV).

3.4.7. Conclusions on the comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials were appropriate to support the comparative analysis.

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics, between MON 94100 oilseed rape and the conventional counterpart needs further assessment for environmental safety.
- None of the differences identified in seed composition between MON 94100 oilseed rape and the conventional counterpart needs further assessment regarding food and feed safety except for the levels of carbohydrates (not treated), calcium (treated) and ADF (both not treated and treated), which are further assessed in Section 3.5.

3.5. Food/feed safety assessment[16]

3.5.1. Effects of processing

Oilseed rape MON 94100 will undergo existing production processes used for conventional oilseed rape. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM oilseed rape into food and feed products is not expected to result in products being different from those of conventional non-GM oilseed rape varieties.

3.5.2. Stability of the newly expressed protein

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety (EFSA GMO Panel, 2010c, 2011a, 2017, 2021). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown, for example, that when characteristics of known food allergens are examined, a prominent trait attributed to food allergens is protein stability (Helm, 2001; Breiteneder and Mills, 2005; Foo and Mueller, 2021; Costa et al., 2022).

[16] Dossier: Part II – Sections 1.4, 1.5, 1.6, 2; additional information provided: 9/6/2021, 20/8/2021, 19/10/2021, 19/1/2022, 13/5/2022.

3.5.2.1. Effect of temperature and pH on the newly expressed protein

The effects of temperature and pH on the DMO protein have been previously evaluated by the GMO Panel (EFSA GMO Panel, 2013, 2015b, 2019a,b).

3.5.2.2. *In vitro* protein degradation by proteolytic enzymes

The resistance to degradation by pepsin of the newly expressed DMO protein has been previously evaluated by the GMO Panel (EFSA GMO Panel, 2013, 2015b, 2019a,b).

3.5.3. Toxicology

3.5.3.1. Testing of the newly expressed protein

The GMO Panel assessed the safety of the DMO protein expressed in oilseed rape MON 94100 considering molecular characterisation, bioinformatic analyses and the previous assessments of the identical DMO protein expressed in soybean MON 87708 (EFSA GMO Panel, 2013, 2015b, 2019a,b).

The oilseed rape MON 94100 DMO protein has been extensively characterised and the biochemical, structural and functional equivalence with the soybean MON 87708 DMO protein was demonstrated (Section 3.3.3).

Updated bioinformatic analyses revealed no similarities of this DMO protein with known toxins (Section 3.3.2).

No safety concerns for humans and animals were previously identified by the GMO Panel for the DMO protein expressed in soybean MON 87708; a weight-of-evidence approach confirmed the safety of this DMO protein demonstrating it is degraded by proteolytic enzymes, showing no similarity to known toxic and allergenic proteins with bioinformatic analysis and no toxicity was reported in mice in a 28-day study and an acute toxicity study (EFSA GMO Panel, 2013).

Furthermore, the GMO Panel is not aware of any new information that would change previous conclusions on the safety of that DMO protein.

3.5.3.2. Testing of new constituents other than proteins

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the DMO protein have been identified in seed from oilseed rape MON 94100. Therefore, no further food/feed safety assessment of components other than the newly expressed protein is required.

3.5.3.3. Information on altered levels of food and feed constituents

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no altered levels of food/feed constituents have been identified in seeds of oilseed rape MON 94100, except for the levels of carbohydrates, calcium and ADF. These changes are considered not to represent a toxicological concern, considering the biological role of the affected constituent and the magnitude of the changes; therefore, no further toxicological assessment is needed. Further information on the relevance of these findings is provided in Section 3.5.6.

3.5.3.4. Testing of the whole genetically modified food and feed

Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the insert, and no modifications of toxicological concern in the composition of oilseed rape MON 94100 have been identified (Sections 3.3, 3.4 and 3.5.3). Therefore, animal studies on food/feed derived from oilseed rape MON 94100 are not considered necessary (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed diets containing meal (toasted and defatted) derived from oilseed rape MON 94100.

In this study, pair-housed Sprague Dawley Crl:CD[SD] rats (16/sex per group; 2 rats/cage) were allocated to three groups using a randomised complete block design with eight replications.

Groups were fed diets containing oilseed rape MON 94100 meal from plants treated with the intended herbicide (dicamba) at 15% and 5% of inclusion level (the latter supplemented with 10% of the non-GM comparator oilseed rape) and the non-GM comparator (inclusion level 15%).

The study was adapted from OECD test guideline 408 (OECD, 2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and complied with the principles of good

laboratory practice (GLP) with some deviations not impacting the study results and interpretation, which are detailed below.

The stability of the test and control materials was not verified; however, in accordance with 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 MON 94100 in both the GM meal and diets and excluded the presence of the event in the respective controls.

Both GM and control meal and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications for Certified Rodent LabDiet® 5002.

Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance with OECD test guideline 408 (OECD, 2018).

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 MON 94100 is reported in Appendix 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 followings:

- 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 end-points;
- no consistency with increasing dietary incorporation level.

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 MON 94100 meal at 5% or 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 Delaney et al. (2014) propose upper limits for inclusion of oilseed rape meal in rodent diets, the GMO Panel considers that further scientific investigation is required to confirm it.

3.5.4. Allergenicity

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the newly expressed proteins, which is defined as the ability to enhance an allergic reaction.

3.5.4.1. Assessment of allergenicity of the newly expressed protein

A weight-of-evidence approach was followed, taking into account all the information obtained on the newly expressed protein, as no single piece of information or experimental method yield sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU) No 503/2013).

The *dmo* gene encoding for the DMO protein originates from *S. maltophilia*, which is not considered a common allergenic source.

[17] Although animal used in a toxicology study is of the same strain, from the same supplier and is 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).

Updated bioinformatic analyses of the amino acid sequences of the DMO protein, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no relevant similarities to known allergens. The studies on protein stability of the DMO protein have been previously assessed (EFSA GMO Panel, 2013; Section 3.5.2). The allergenic potential of this protein has been previously evaluated (EFSA GMO Panel, 2013, 2015b, 2019a,b). In addition, the GMO Panel did not find an indication that the newly expressed protein DMO at the levels expressed in oilseed rape MON 94100 might be adjuvant.

Furthermore, the applicant provided information on the safety of the DMO protein regarding its potential hazard to cause a celiac disease response. For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the DMO protein identified no perfect or relevant partial matches with known celiac disease peptide sequences.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed DMO protein in oilseed rape MON 94100 may be allergenic.

3.5.4.2. Assessment of allergenicity of the whole GM plant

The GMO Panel regularly reviews the available publications on food allergy to oilseed rape. However, to date, oilseed rape is not considered a common allergenic food[18] (OECD, 2001). Therefore, the GMO Panel does not request experimental data to analyse the allergen repertoire of GM oilseed rape.

In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed protein (see Sections 3.3, 3.4 and 3.5), the GMO Panel identifies no indications of a potentially increased allergenicity of food and feed derived from oilseed rape MON 94100 with respect to that derived from its conventional counterpart.

3.5.5. Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to DMO protein newly expressed in MON 94100 oilseed rape. Dietary exposure was estimated based on protein expression levels reported in this application for MON 94100 oilseed rape treated with the intended herbicide dicamba, the current available consumption data and feed practices, the foods and feeds currently available on the market and the described processing conditions.

For the purpose of estimating dietary exposure, the levels of DMO protein in MON 94100 oilseed rape seeds were derived from replicated field trials (four replicates from five locations, n = 20) in 2018 in the United States and Canada. Table 1 in Section 3.3.4 shows the protein expression levels used to estimate both human and animal dietary exposure.

3.5.5.1. Human dietary exposure

Currently, oil is almost the only food commodity derived from oilseed rape regularly consumed by the European population, although other food commodities derived from oilseed rape have been approved as novel food in recent years, e.g. protein isolates (EFSA NDA Panel, 2013) and oilseed rape powder (EFSA NDA Panel, 2020). Different preparations of protein isolates from oilseed rape meal are found under commercial names. Currently, both protein isolates and oilseed rape powder seem to be absent in the European market as these products were not found in Mintel's Global New Products Database.[19] In addition, no consumption data for protein isolates and meat imitates from oilseed rape have been reported in the EFSA Comprehensive European Food Consumption Database (EFSA consumption database).[20] However, following an overly conservative approach, the applicant provided estimates of the dietary exposure to DMO protein making use of the available consumption data on

[18] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[19] The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.5 million food and beverage products of which more than 1,000,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 24 of its 27 member countries and Norway presented in the Mintel GNPD.

[20] https://www.efsa.europa.eu/en/applications/gmo/tools. Data accessed: February 2021.

meat imitates and protein supplements in the EFSA consumption database assuming they are derived from MON 94100 oilseed rape.

Human dietary exposure was estimated across different European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the quantity of seeds in the consumed commodities before assigning newly expressed protein levels to the relevant commodities.[21] No losses in the newly expressed proteins during processing were considered.

The highest chronic and acute dietary exposure to DMO protein (high consumers) was estimated as 9.7 μg/kg body weight (bw) per day and 14.9 μg/kg bw per day, respectively, in both cases in the age class 'Adults' via the consumption of 'Protein and protein components for sports people'.

Additional dietary exposure to DMO protein might occur via the consumption of pollen supplements under the assumption that these supplements contain pollen from MON 94100 oilseed rape. Consumption data on pollen supplements are available for a few consumers across eight different European countries.[22] However, since no data on the presence of newly expressed proteins in pollen were available, the potential dietary exposure to DMO protein from the consumption of pollen supplements could not be estimated.

3.5.5.2. Animal dietary exposure

Dietary exposure to DMO protein in oilseed rape MON 94100 was estimated across different animal species, as below described, assuming the consumption of oilseed rape products commonly entering the feed supply chain (i.e. oilseed rape meal). A conservative scenario with 100% replacement of conventional oilseed rape products by the oilseed rape MON 94100 products was considered.

Mean levels (dry weight) of the newly expressed protein in seeds from oilseed rape MON 94100 treated with the intended herbicide used for dietary exposure are listed in Table 1 (Section 3.3.4).

Mean levels of newly expressed protein in oilseed rape meal were calculated to be, respectively, 1.6-fold higher than in seed, based on adjusting factors that take into account the protein content in this feed material relative to oilseed rape seed (OECD, 2001), and assuming that no protein is lost during processing.

The applicant estimated dietary exposure to the DMO protein via the consumption of oilseed rape meal in broiler, finishing pig and lactating dairy cow, based on default values for animal body weight, daily feed intake and inclusion rates (percentage) of oilseed rape meal in rations, as provided for the EU by OECD (2009). Estimated dietary exposure in the concerned animals is reported in Table 5.

Table 5: Dietary exposure to DMO protein (μg/kg bw per day) in livestock, based on the consumption of oilseed rape meal

	Body weight (kg)	TDI feed (kg DM/animal)	IR (%)	DMO (μg/kg bw per day)
Broiler	1.7	0.12	18	13
Finishing pig	100	3	20	6
Dairy cow	650	25	10	4

3.5.6. Nutritional assessment of GM food and feed/endogenous constituents

The intended trait of MON 94100 oilseed rape is herbicide tolerance with no intention to alter nutritional parameters. However, in MON 94100 oilseed rape, the levels of carbohydrates (in not treated plants with the intended herbicide), calcium (treated) and ADF (both not treated and treated) were significantly different from the non-GM comparator and showed a lack of equivalence with the set of non-GM reference varieties (Section 3.4.7). The biological relevance of these compounds, the

[21] Example: assuming around 15–20% protein content in the meat imitates and 25% protein content in seeds, 100 g of 'Textured soy protein' (meat imitate) would contain approximately 60 g of seeds. This results in a concentration of 0.35 μg of DMO protein per gram of meat imitate as compared to the 0.59 μg/g reported as mean concentration in the seeds.

[22] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database. Data accessed: March 2022.

role of MON 94100 oilseed rape as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.6.1. Human nutrition

The main food commodity derived from oilseed rape regularly consumed by the European population is oil, which is typically devoid of carbohydrates, fibre and minerals. As commented in Section 3.5.5.1, the presence in the European market of other oilseed rape derived food commodities such as protein isolates and oilseed rape powder seems to be currently absent; however, the nutritional assessment also considered these commodities.

The relatively small decrease in carbohydrates (< 2%) is not relevant for human nutrition considering also their absence in the oil and their low concentration in protein isolates; the same conclusion applies to oilseed rape powder even if it contains a relatively high content of carbohydrates (~ 35%).

In the context of human nutrition, fibre is referred to as dietary fibre, which primarily includes non-starch polysaccharides (mainly cellulose, hemicelluloses, pectins and other hydrocolloids) and lignin (EFSA NDA Panel, 2010). Therefore, the observed decrease (~ 5%) in ADF (cellulose and lignin) implies a decreased intake of dietary fibre. While protein isolates typically contain very small amounts of fibre, this is one of the main components of oilseed rape powder (EFSA NDA Panel, 2013, 2020). The relatively small decrease of ADF reported in seeds from MON 94100 oilseed rape is unlikely to represent any nutritional concern for humans.

Dietary reference values are set for calcium as it is involved in many physiological functions (EFSA NDA Panel, 2015). Foods rich in calcium are dairy products, dark green vegetables, legumes, nuts, fish with soft bones (e.g. canned sardines) and calcium-fortified foods. Protein isolates and oilseed rape powder should not be considered typical sources of this mineral. Therefore, the decrease (~ 6%) in the levels of calcium in seeds from oilseed rape MON 94100 does not raise any nutritional concern.

3.5.6.2. Animal nutrition

Oilseed rape is mainly a source of protein and lipids, while the percentage of carbohydrate is limited, and the main carbohydrate is represented by NDF, that is the total cell wall which is comprised of the ADF fraction plus hemicellulose. The decrease of carbohydrate in GM not treated oilseed rape compared to the conventional counterpart is not an issue for animal nutrition.

The decrease of ADF percentage in seeds of the GM oilseed rape, compared to the conventional counterpart, is linked to the decrease of carbohydrates percentage, and does not represent a problem for animal nutrition.

Diets for animals are usually balanced for the content of major minerals, including calcium, and eventually supplemented when the amount provided by feed is not enough to satisfy nutritional requirements. The observed decrease of calcium in seeds of the GM oilseed rape compared to the conventional counterpart does not pose an issue for animals.

3.5.7. Post-market monitoring of GM food and feed

The GMO Panel concluded that oilseed rape MON 94100, as described in this application, does not raise any nutritional concern and is as safe as the conventional counterpart and the non-GM reference varieties tested. Therefore, the GMO Panel considers that post-market monitoring of food and feed from this GM oilseed rape is not necessary.

3.5.8. Conclusion on the food and feed safety assessment

The GMO Panel did not identify indications of safety concerns regarding toxicity, allergenicity or adjuvanticity related to the presence of the newly expressed protein in oilseed rape MON 94100. The GMO Panel found no evidence that the genetic modification impacts the overall safety of oilseed rape MON 94100. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of oilseed rape MON 94100 does not represent any nutritional concern, in the context of this application. The GMO Panel concludes that oilseed rape MON 94100 does not represent a nutritional concern, and is as safe as, the conventional counterpart and non-GM oilseed rape reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

3.6. Environmental risk assessment and monitoring plan[23]

3.6.1. Environmental risk assessment

Considering the scope of application EFSA-GMO-NL-2020-169, which excludes cultivation, the environmental risk assessment (ERA) of oilseed rape MON 94100 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable oilseed rape MON 94100 seeds during transportation and/or processing (EFSA GMO Panel, 2010a).

3.6.1.1. Persistence and invasiveness of the GM plant

Oilseed rape (*Brassica napus*) is an annual allotetraploid species (2n = 38, genome constitution AACC), which has probably evolved through hybridisation and polyploidisation between the two diploid species *Brassica rapa* (2n = 20, AA) and *Brassica oleracea* (2n = 18, CC). Oilseed rape seeds have the ability to survive in soils for several years (Lutman et al., 2004, 2005, 2008; Messéan et al., 2007; D'Hertefeldt et al., 2008; Gruber et al., 2008; Beckie and Warwick, 2010; Peltonen-Sainio et al., 2014; Belter, 2016) and demographic studies and surveys have shown the ability of oilseed rape (*B. napus*) seed to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries (e.g. Devos et al., 2012; Bauer-Panskus et al., 2013; COGEM, 2013; Banks, 2014; Hecht et al., 2014; Schulze et al., 2014; Katsuta et al., 2015; Bailleul et al., 2016; Busi and Powles, 2016; Franzaring et al., 2016; Nishizawa et al., 2016; Pandolfo et al., 2016). Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites (e.g. mowed areas, field edges) to germinate and capture resources rapidly. In undisturbed natural habitats, oilseed rape lacks the ability to establish stable populations over successive years, possibly due to the absence of competition-free germination sites (Crawley et al., 1993, 2001; Meffin et al., 2015) and exposure to biological and abiotic stressors likely limiting fitness (COGEM, 2013; Busi and Powles, 2016). Once established in competition-free germination sites, feral populations decline over a period of years (Crawley and Brown, 1995, 2004; Knispel et al., 2008; Squire et al., 2011; Banks, 2014; Busi and Powles, 2016). However, if habitats are disturbed on a regular basis, then feral populations can persist for longer periods (Claessen et al., 2005a,b; Garnier et al., 2006). The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, to recruitment from seed emerging from the soil seedbank or shed by resident feral adult plants or to redistribution of feral seed from one location to another (Pivard et al., 2008a,b; Bailleul et al., 2016).

It is unlikely that the intended trait of oilseed rape MON 94100 will provide a selective advantage to oilseed rape plants, except when they are exposed to dicamba-containing herbicides. Should these plants be exposed to such herbicides, their abundance may increase locally (Londo et al., 2010, 2011; Watrud et al., 2011), allowing the establishment of transient populations. However, the likelihood of such an event will be restricted to managed environments, which may occasionally be treated with such herbicides. Moreover, this fitness advantage will not allow oilseed rape MON 94100 to overcome other biological and abiotic factors (described above) limiting a plant's persistence and invasiveness.

In conclusion, the GMO Panel considers that oilseed rape MON 94100 will be equivalent to conventional oilseed rape varieties in their ability to survive and establish feral populations under European environmental conditions in case of accidental release into the environment of viable oilseed rape MON 94100 seeds.

3.6.1.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA or through vertical gene flow via cross-pollination from feral plants originating from spilled seeds.

Plant-to-microorganism gene transfer

Genomic DNA can be a component of food and feed products derived from oilseed rape. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals,

[23] Dossier: Part II – Sections 5 and 6; additional information provided: 13/5/2022.

and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

Homologous recombination is known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

In addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end joining and microhomology-mediated end joining are theoretically possible (Hülter and Wackernagel, 2008; EFSA, 2009). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a theoretical HGT would provide to bacterial recipients in the environment. The bioinformatic analysis for event MON 94100 revealed no homology with known DNA sequences from bacteria which would facilitate homologous recombination.

In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from oilseed rape MON 94100 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

Plant-to-plant gene transfer

The potential of feral GM oilseed rape plants originating from seed import spills to transfer recombinant DNA to sexually cross-compatible plants and the environmental consequences thereof were considered.

For plant-to-plant gene transfer to occur, imported GM oilseed rape seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated oilseed rape with synchronous flowering and environmental conditions favouring cross-pollination.

Oilseed rape is an open pollinating crop plant capable of cross-pollinating with other *Brassica* crops (Eastham and Sweet, 2002). It can also spontaneously hybridise with sexually compatible feral and wild relatives. Several hybrids between oilseed rape and wild relatives have been reported in the scientific literature.

Evidence suggests that transgenes could readily hybridise with different wild *Brassica* relatives (Ellstrand et al., 1999; FitzJohn et al., 2007) and introgress in *B. rapa*, *B. juncea* and *B. oleracea* but introgression is expected to be rare with *B. nigra*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapis arvensis* (reviewed by FitzJohn et al., 2007; Devos et al., 2009; Liu et al., 2013). Of significance, transgene introgression has only been confirmed under field conditions for *B. rapa* (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Jørgensen et al., 2004; Norris et al., 2004; Jørgensen, 2007).

For transgene introgression to occur, feral GM oilseed rape must require some overlap in flowering in time and space with compatible relatives. Subsequently, transgenes must be transmitted through successive backcross generations or selfing, so that they become stabilised into the genome of the recipient (de Jong and Rong, 2013; Garnier et al., 2014). Because of these barriers (Luijten et al., 2015), reported incidences of hybrids and backcrosses with *B. rapa* were found to be low in fields (Jørgensen et al., 2004; Norris et al., 2004; Warwick et al., 2008; Elling et al., 2009), or at ports, along roadsides and riverbanks (Saji et al., 2005; Aono et al., 2006, 2011; Yoshimura et al., 2006; Elling et al., 2009; Katsuta et al., 2015; Luijten et al., 2015).

The GMO Panel does not consider the occurrence of feral oilseed rape MON 94100 plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, as there is no evidence that the trait will enhance the vertical gene flow potential, or fitness, persistence or invasiveness of feral oilseed rape MON 94100, or cross-compatible plants such as hybridising wild relatives. However, when exposed to dicamba- and/or dicamba-containing herbicides, occasional cross-compatible plants that acquired the herbicide tolerance trait through vertical gene flow are likely to exhibit a selective advantage, which may lead to their increased abundance. The likelihood of such an event to happen will be restricted to managed environments, which may occasionally be treated with such herbicides, so that environmental impacts will be minimal. Therefore, the GMO Panel considers that the acquisition of the herbicide tolerance trait by cross-compatible plants would not create additional environmental impacts.

In conclusion, the GMO Panel considers that the likelihood of environmental effects as a consequence of the spread of genes from oilseed rape MON 94100 in Europe will not differ from that of conventional oilseed rape varieties.

3.6.1.3. Interactions of the GM plant with target organisms

Taking the scope of application EFSA-GMO-NL-2020-169 (no cultivation) and thus the absence of target organisms into account, potential interactions of feral oilseed rape MON 94100 plants arising from seed import spills with target organisms are not considered a relevant issue.

3.6.1.4. Interactions of the GM plant with non-target organisms

Given that environmental exposure of non-target organisms to spilled GM seeds or feral GM oilseed rape plants arising from spilled oilseed rape MON 94100 seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM oilseed rape, potential interactions of the oilseed rape MON 94100 with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern.

3.6.1.5. Interactions of the GM plant with abiotic environment and biogeochemical cycles

Given that environmental exposure to spilled seeds or feral oilseed rape MON 94100 plants arising from seed import spills is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM oilseed rape, potential interactions with the abiotic environment and biogeochemical cycles are not considered by the GMO Panel to raise any environmental safety concern.

3.6.2. Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA are correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus, a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from oilseed rape MON 94100, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for oilseed rape MON 94100 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by CropLife Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis and a final report at the end of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of oilseed rape MON 94100. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

3.6.3. Conclusion on the environmental risk assessment and monitoring plan

The GMO Panel concludes that it is unlikely that oilseed rape MON 94100 would differ from conventional oilseed rape varieties in its ability to persist under European environmental conditions. Considering the scope of application EFSA-GMO-NL-2020-169, interactions of feral oilseed rape MON 94100 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from oilseed rape MON 94100 to bacteria does not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the agronomic and phenotypic analysis, the routes and levels of exposure, the GMO Panel concludes that oilseed rape MON 94100 would not raise safety concerns in the event of accidental release of viable GM oilseed rape seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of oilseed rape MON 94100. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

4. Overall conclusions

The GMO Panel was asked to carry out a scientific assessment of oilseed rape MON 94100 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that oilseed rape MON 94100 contains a single insert consisting of one copy of the *dmo* expression cassette. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance to the requirements listed in the EFSA Technical Note. Updated bioinformatic analyses of the sequences encoding the newly expressed protein and other ORFs present within the insert or spanning the junctions between the insert and genomic DNA do not raise any safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the DMO (including DMO + 27) protein is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of the oilseed rape MON 94100-produced DMO and DMO + 27 indicate that they are equivalent to their soybean counterparts. Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between oilseed rape MON 94100 and its conventional counterpart needed further assessment, except for the levels of carbohydrates, calcium and ADF in seeds, which do not raise nutritional and safety concerns. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the DMO protein as expressed in oilseed rape MON 94100. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of oilseed rape MON 94100. In the context of this application, the consumption of food and feed from oilseed rape MON 94100 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that oilseed rape MON 94100 is as safe as the conventional counterpart and non-GM oilseed rape reference varieties tested, and no post-market monitoring of food/feed is considered necessary. The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from oilseed rape MON 94100 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of oilseed rape MON 94100. Based on the relevant records identified through the literature searches, the GMO Panel does not identify any safety issues pertaining to the uses of oilseed rape MON 94100. The GMO Panel concludes that oilseed rape MON 94100 is as safe as its conventional counterpart and the tested non-GM oilseed rape reference varieties with respect to potential effects on human and animal health and the environment.

5. Documentation as provided to EFSA

- Letter from the Competent Authority of The Netherlands received on 16th November 2020 concerning a request for authorization of the placing on the market of genetically modified oilseed rape MON 94100 submitted in accordance with Regulation (EC) No 1829/2003 by Bayer Agriculture BV (EFSA Ref. EFSA-GMO-NL-2020-169; EFSA-Q-2020-00749).
- The application was made valid on 25th March 2021.
- Additional Information (Clock 1) was requested on 9th April 2021.
- Additional Information (Clock 1) was received on 9th June 2021.
- Additional Information (Clock 2) was requested on 21st April 2021 (EURL).
- Additional Information (Clock 2) was received on 23rd April 2021.
- Additional Information (Clock 3) was requested on 21st June 2021.
- Additional Information (Clock 3) was received on 20th August 2021.
- Additional Information (Clock 4) was requested on 17th August 2021.
- Additional Information (Clock 4) was received on 19th October 2021.
- Additional Information (Clock 5) was requested on 19th November 2021.
- Additional Information (Clock 5) was received on 19th January 2021 partial; 19th April 2022 complete.
- Additional Information (Clock 6) was requested on 21st March 2022.
- Additional Information (Clock 6) was received on 13th May2022 partial; 24th May 2022 complete.
- Supplementary information was provided on voluntary basis on 19th October 2021 and on 13th May 2022.

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Abbreviations

ADF	acid detergent fibre
bp	base pair
bw	body weight
DMO	dicamba mono-oxygenase
dw	dry weight
ELISA	enzyme-linked immunosorbent assay
ERA	environmental risk assessment
FMV	figwort mosaic virus
fw	fresh weight
GLP	good laboratory practice
GMO	genetically modified organism
HGT	horizontal gene transfer
HR	homologous recombination
JSA	junction sequence analysis
LB	left border
MS	mass spectrometry
NGS	next-generation sequencing
NDF	neutral detergent fibre
Nos	nopaline synthase
OECD	Organisation for Economic Co-operation and Development
ORF	open reading frame
PClSV	peanut chloritic streak caulimovirus
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
rbcS	Rubisco
RB	right border
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
T-DNA	transfer-deoxyribonucleic acid
TEV	tobacco etch virus
Usp	unknown seed protein
UTR	untranslated region

Appendix A – Statistical analysis and statistically significant findings in the 90-day toxicity study in rats on oilseed rape MON 94100

A.1. Statistical analysis of the 90-day study on oilseed rape MON 94100 in rats

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

The main statistical analysis compared rats consuming high- and low-dose test diets with those consuming the control diet. The statistical analysis of continuous endpoints was performed using linear mixed models, applied separately for each parameter and period. For food consumption data (with cage-based observations), the model included treatment, sex and treatment-by-sex interaction as fixed effects; replicate-within-sex was the random effect. For body weight data, organ weights (absolute and relative), clinical pathology parameters and FOB evaluations (all with individual-level observations), the fixed effects were treatment, sex and treatment-by-sex interaction; the random effects were replicate-within-sex and the interaction of replicate and dose within sex (the latter representing the cage effect). For locomotor activity data, the model was expanded to include time interval as an additional fixed effect and terms for the interaction of time interval with all the other factors. For all the models, in case the sex-by-treatment interaction was significant (and in any case for sex-specific parameters), a sex-specific analysis was performed. For categorical parameters (microscopic findings), the high-dose and control groups were compared with Fisher's exact test.

Historical control data were provided for organ weight, clinical pathology and motor activity parameters, and used to assess statistical differences identified for such parameters in the study. Missing data were considered by the Panel and found not impacting the results (Table A.1).

Table A.1: Statistically significant findings in 90-day study on oilseed rape MON 94100 in rats

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Ambulatory activity	Total counts increased (25%) in females of the high-dose group	Within normal variation for this parameter. Not an adverse effect of treatment.
Haemoglobin, haematocrit and red blood cell count	Reduced (3%) in the high-dose groups.	Low magnitude. Not an adverse effect of treatment.
Monocyte count	Decreased (20%) in both test groups (significant at the low dose only)	Within normal variation for this parameter. Not an adverse effect of treatment.
Neutrophil count	Decreased (25%) at the low dose	Within normal variation for this parameter. No dose response. Not an adverse effect of treatment.
Testes weight	Increased 6% at the low dose	Small magnitude. No associated histopathological findings. Not an adverse effect of treatment.
Uterus weight relative to body weight	Increased 28% at the high dose and 33% at the low dose. Statistically significant at low dose only.	Within normal variation. The increase is mainly driven by animals with uterine dilatation/high fluid content, which is typically related to oestrus cycling. The incidence of uterine dilatation/high fluid content is not statistically different from concurrent control values and is within normal variation. Not an adverse effect of treatment.
Adrenal weights (absolute and relative to body weight)	Increased (10%) at the low dose.	Small magnitude. No dose response. No associated histopathological findings. Not an adverse effect of treatment.

Statistically significant parameter/endpoint	Finding	GMO Panel interpretation
Heart weights relative to body weight	Increased (10%) in both groups.	Small magnitude. No associated histopathological findings (controls have high level of necrosis). Not an adverse effect of treatment.
Liver weights relative to body weight	Increased (10%) in both groups.	Small magnitude, within normal variation. No associated histopathological or clinical chemistry findings. Not an adverse effect of treatment.
Pituitary weight	Increased (10%) at the low dose.	Small magnitude. No dose response. No associated histopathological findings. Not an adverse effect of treatment.