Adopted: 17 September 2025

DOI: 10.2903/j.efsa.2025.9692

efsa JOURNAL

SCIENTIFIC OPINION

Assessment of genetically modified oilseed rape LBFLFK (application EFSA-GMO-DE-2019-157)

EFSA Panel on Genetically Modified Organisms (GMO) | Josep Casacuberta | Francisco Barro | Albert Braeuning | Ruud de Maagd | Michelle M. Epstein | Thomas Frenzel | Jean-Luc Gallois | Frits Koning | Antoine Messéan | F. Javier Moreno | Fabien Nogué | Giovanni Savoini | Alan H. Schulman | Christoph Tebbe | Eve Veromann | Michele Ardizzone | Martina Bonatti | Giacomo De sanctis | Silvia Federici | Antonio Fernandez Dumont | Arianna Ferrari | Andrea Gennaro | Tilemachos Goumperis | Dafni Maria Kagkli | Paolo Lenzi | Aleksandra Lewandowska | Ana M. Camargo | Franco Maria Neri | Nikoletta Papadopoulou | Pietro Piffanelli | Tommaso Raffaello | Reinhilde Schoonjans | José Ángel Gómez Ruiz

Correspondence: nif@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

Application EFSA-GMO-DE-2019-157 (oilseed rape LBFLFK) was submitted to EFSA to be risk assessed as full-scope, i.e. for import, processing, and food and feed uses, within the EU. Oilseed rape LBFLFK was developed to alter its fatty acid profile and to confer tolerance to imidazolinone herbicides. Oilseed rape LBFLFK contains two inserts, not genetically linked, each consisting of a single copy of the T-DNA, which contains 13 expression cassettes (coding for 11 unique proteins). The Panel on Genetically Modified Organisms (GMO Panel) was unable to conclude on the safety of oilseed rape LBFLFK when considering the full-scope of this application. The safety of the elongases and desaturases newly expressed in oilseed rape LBFLFK cannot be established. Similarly, the safety and nutritional adequacy of the oil and full-fat seeds derived from oilseed rape LBFLFK for its use in terrestrial farmed and companion animals and in aquaculture cannot be established. However, the GMO Panel concludes that the consumption of refined, bleached and deodorised (RBD) oil from oilseed rape LBFLFK is safe and does not represent any nutritional concern in humans under the conditions of use proposed by the applicant. Considering the potential safety concerns, LBFLFK oil should not be used for high-temperature applications. Post-market monitoring is appropriate to confirm the expected consumption and the application of conditions of uses in humans. Furthermore, the GMO Panel did not identify any environmental concerns related to increased persistence and invasiveness or to any potential for gene transfer from oilseed rape LBFLFK, and concludes that it poses no risks for terrestrial non-target organisms (NTOs) or biogeochemical cycles in this environment. The GMO Panel cannot conclude on the safety of oilseed rape LBFLFK to NTOs or biogeochemical cycles in the aquatic environment. Based on this, the GMO Panel is unable to conclude on the adequacy of the post-market environmental monitoring plan proposed by the applicant.

KEYWORDS

AHAS, desaturases, elongases, fatty acid profile, genetic engineering, GM, import and processing, LBFLFK, oil, oilseed rape (*Brassica napus* AACC)

CONTENTS

SUMMARY

Following the submission of application EFSA-GMO-DE-2019-157 under Regulation (EC) No 1829/2003 from BASF Plant Science Company GmbH (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA, referred to hereafter as 'GMO Panel') was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide-tolerant oilseed rape (*Brassica napus* AACC) LBFLFK according to Regulation (EU) No 503/2013. The fatty acid profile in oilseed rape LBFLFK is altered due to the presence of several newly expressed elongases and desaturases. The scope of application EFSA-GMO-DE-2019-157 is for import, processing, and food and feed uses within the EU of oilseed rape LBFLFK (referred to hereafter as 'full-scope application'), and does not include cultivation in the EU.

The GMO Panel conducted a comprehensive assessment of oilseed rape LBFLFK under a full-scope application, following the current risk assessment approach in accordance with Regulation (EU) No 503/2013. A full-scope application requires a risk assessment to cover all potential uses of oilseed rape LBFLFK, i.e. import, processing, and food and feed uses, mirroring the range of uses applicable to conventional oilseed rape. However, in this particular case, the assessment could not be conducted as a standard full-scope application for several reasons:

a. The majority of newly expressed proteins in oilseed rape LBFLFK are membrane bound and, because of this, technically difficult to isolate, characterise or test according to current requirements (Regulation (EU) No 503/2013). Furthermore, a history of safety use for consumption (HoSU) could not be documented for these proteins;
b. The substantially different composition in the fatty acid profile of oilseed rape LBFLFK as compared to the conventional counterpart requires a modification of the standard approach usually followed to nutritionally assess GM crops. The uses of oilseed rape LBFLFK, due to its particular composition in fatty acids, will differ entirely from those of its conventional counterpart. This composition determines new uses (intended uses) that must first be identified and then considered during the assessment, e.g. its use as a potential source of C20:5,ω3 (eicosapentaenoic acid, EPA) and C22:6,ω3 (docosahexaenoic acid, DHA);
c. Furthermore, available data indicate that the fatty acid profile of refined, bleached and deodorised (RBD) LBFLFK oil may substantially vary depending on several biological factors, including the genetic background of the oilseed rape plant. This reported high variability limits the extent to which the current compositional data set can be considered representative for a full-scope assessment, and adds further complexity to the assessment.

The molecular characterisation data establish that oilseed rape LBFLFK contains two inserts (Insert 1 and Insert 2), not genetically linked, each consisting of a single copy of the T-DNA originating from the same transformation vector, which contains 13 expression cassettes (coding for 11 unique proteins). The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and complies with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the newly expressed proteins (NEPs), the sequences corresponding to open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise safety concerns. The stability of the inserted DNA and of the introduced trait conferring herbicide tolerance and an altered fatty acid composition is confirmed over several generations. The methodology used to quantify the levels of the NEPs is considered adequate. As most of the NEPs could not be produced in a heterologous expression system in the amounts and purity sufficient for the protein characterisation, the description of biochemical, structural and functional properties of the NEPs was performed with a membrane fraction purified from crude extracts of developing embryos that were isolated from immature seeds of oilseed rape LBFLFK (plant-produced proteins, PPP). The activities of all the introduced elongases and desaturases for the intended substrates were demonstrated upon expression in yeast. However, several protein characterisation assays failed for a number of NEPs as PPP. These include protein detection using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and/or western blotting that was not successful for D6E(*Pp*) and O3D(*Pi*), and the absence of detectable enzyme activity specific for D6D(*Ot*), D6E(*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D4D(*Pl*) and D4D(Tc). Moreover, based on the provided data, partial glycosylation of D12D(*Ps*), D6D(*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), D5E(*Ot*) and D4D(*Tc*) cannot be excluded. Due to failure of some of the analyses, the GMO panel cannot conclude that a membrane fraction purified from crude extracts of developing embryos is an appropriate source of NEP for the safety studies.

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 differences identified in agronomic and phenotypic characteristics between oilseed rape LBFLFK and the non-GM comparator needed further assessment except for germination of harvested seeds, which underwent additional evaluation and was found not to raise environmental concerns.

The GMO Panel was unable to conclude on the safety of oilseed rape LBFLFK when considering the full-scope of the application. The GMO Panel conducted a protein safety assessment of the newly expressed proteins in oilseed rape LBFLFK based on the information provided by the applicant. The GMO Panel concludes that the safety of the elongases and desaturases newly expressed in oilseed rape LBFLFK cannot be established. The AHAS protein, also newly expressed in oilseed rape LBFLFK, does not raise safety concerns for human and animal health. In the case of the human nutritional assessment, the applicant provided detailed information on the intended uses of the RBD oil, and submitted a list of food products with a predefined composition of relevant fatty acids (see Appendix C). Based on this information, the GMO Panel concludes

that the consumption of RBD LBFLFK oil is safe and does not represent any nutritional concern in humans under the conditions of use proposed by the applicant and considered during the pre-market risk assessment (see Appendix C). If these conditions of use would change in future (foods and maximum fatty acid concentrations in these foods), the current nutritional assessment will have to be revisited.

A post market monitoring (PMM) plan, in line with Article 6(5)(e) of Regulation (EC) No 1829/2003 and as indicated in Regulation (EU) No 503/2013, is recommended to confirm the predicted consumption and the application of conditions of use of LBFLFK oil in food considered during the pre-market risk assessment. The GMO Panel considers that the proposed labelling is not scientifically correct as it does not fully characterise the compositional changes in the GM crop. Therefore, the labelling should be adjusted to be scientifically correct. Furthermore, considering the potential safety concerns identified for frying or other high-temperature uses of oils rich in polyunsaturated fatty acids (PUFAs), appropriate labelling of specific food uses of RBD LBFLFK oil (e.g. margarine, butter, oil; see Appendix C for details) could help ensure that these foods are not used for high-temperature applications.

In animals, based on the results of the comparative compositional analysis, the nutritional assessment and the available feeding studies in aquatic species, the GMO Panel concludes that the safety and nutritional adequacy of the oil and full-fat seeds derived from oilseed rape LBFLFK for its use in terrestrial farmed and companion animals and in aquaculture cannot be established. Consequently, it is also not in a position to provide comments on the post-market monitoring of the GM feed.

The GMO Panel did not identify any environmental concerns related to increased persistence and invasiveness or to any potential for gene transfer from oilseed rape LBFLFK. Likewise, the GMO Panel concludes that oilseed rape LBFLFK poses no risks for terrestrial non-target organisms (NTOs) or biogeochemical cycles in this environment, whereas the GMO Panel cannot conclude on the safety of oilseed rape LBFLFK to NTOs or biogeochemical cycles in the aquatic environment. Owing to the inconclusiveness of the environmental risk assessment for some areas of concern and environments, the GMO Panel is unable to conclude on the adequacy of the post-market environmental monitoring (PMEM) plan proposed by the applicant.

The GMO Panel cannot conclude on the safety of oilseed rape LBFLFK when considering the full-scope of the application. On the other hand, the GMO Panel concludes that the consumption of RBD LBFLFK oil is safe and does not represent any nutritional concern for humans under the conditions of use proposed by the applicant and considered during the pre-market risk assessment.

1 | INTRODUCTION

The scope of the application EFSA-GMO-DE-2019-157 is for food and feed uses, import and processing of oilseed rape LBFLFK and does not include cultivation in the European Union (EU). Oilseed rape LBFLFK was developed to confer tolerance to imidazolinone herbicides and to alter the fatty acid profile (increased C20:5ω3 (eicosapentaenoic acid, EPA) and C22:6ω3 (docosahexaenoic acid, DHA)) in order to be used in food and feed.

1.1 | Background

On 20 June 2019, the European Food Safety Authority (EFSA) received from the Competent Authority of Germany application EFSA-GMO-DE-2019-157 for authorisation of oilseed rape LBFLFK (Unique Identifier BPS-BFLFK-2), submitted by BASF Plant Science Company GmbH (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application EFSA-GMO-DE-2019-157, EFSA informed EU Member States and the European Commission (EC) 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 (EU) No 503/2013,[3] with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 27 November 2019, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-DE-2019-157. Such time limit was extended whenever EFSA and/or 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 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 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-DE-2019-157 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 LBFLFK in the context of its scope as defined in application EFSA-GMO-DE-2019-157.

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 assessment of oilseed rape LBFLFK on the valid application EFSA-GMO-DE-2019-157, additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by EU Member States and relevant peer-reviewed- scientific publications. As part of this comprehensive information package, the GMO Panel received additional unpublished studies submitted by the applicant in order to comply with the specific provisions of Regulation (EU) No 503/2013.[3] A list of these additional unpublished studies is provided in Appendix A.

2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 1829/2003, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017a, 2017b, 2018, 2019a, 2019b; EFSA GMO Panel, 2010b) for the risk assessment of

[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/question/EFSA-Q-2019-00394.

[3]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorization 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 at: https://open.efsa.europa.eu/question/EFSA-Q-2019-00394.

GM plants. For this application, the contractors performed preparatory work for the evaluation of the applicants' literature search (OC/EFSA/GMO/2018/04), the bioinformatic analyses (OC/EFSA/GMO/2021/06) and methods applied for the statistical analysis of the field trial data (OC/EFSA/GMO/2018/02 Lot 1) and the analysis of the 90-day toxicity study on oilseed rape LBFLFK (OC/EFSA/GMO/2018/02 Lot 2; EOI/EFSA/SCIENCE/2020/01 – CT02GMO).

3 | ASSESSMENT

3.1 | Introduction

The fatty acid metabolic pathway in conventional oilseed rape produces C18:1ω9 (oleic acid, OLA) in plastids. OLA is transported to the endoplasmic reticulum, where it becomes a substrate of delta-12-desaturase leading to the production of C18:2ω6 (linoleic acid) that is converted by delta-6-desaturase to C18:3ω3 (linolenic acid). The introduction of the seven desaturases and three elongases to develop oilseed rape LBFLFK (full list in Table 1) allows production of DHA and its biosynthetic intermediate EPA from these endogenous fatty acids through an aerobic pathway (Senger et al., 2016; Figure 1).

TABLE 1 Summary of newly expressed proteins (NEPs) in oilseed rape LBFLFK, including their abbreviations and basic information to facilitate identification and cross-reference throughout the document.

Enzyme full name	Enzyme abbreviation	Coding sequence designation	Donor organism
Delta-12 desaturase (Ps)	D12D(*Ps*)	*c-D12D(Ps)*	*Phytophthora sojae*
Delta-6 desaturase (Ot)	D6D(*Ot*)	*c-D6D(Ot)*	*Ostreococcus tauri*
Delta-6 elongase (Tp)	D6E(*Tp*)	*c-D6E(Tp)*	*Thalassiosira pseudonana*
Delta-6 elongase (Pp)	D6E(*Pp*)	*c-D6E(Pp)*	*Physcomitrella patens*
Delta-5 desaturase (Tc)	D5D(*Tc*)	*c-D5D(Tc)*	*Thraustochytrium* sp.
Omega-3 desaturase (Pir)	O3D(*Pir*)	*c-O3D(Pir)*	*Pythium irregulare*
Omega-3 desaturase (Pi)	O3D(*Pi*)	*c-O3D(Pi)*	*Phytophthora infestans*
Delta-5 elongase (Ot)	D5E(*Ot*)	*c-D5E(Ot)*	*Ostreococcus tauri*
Delta-4 desaturase (Tc)	D4D(*Tc*)	*c-D4D(Tc)*	*Thraustochytrium* sp.
Delta-4 desaturase (Pl)	D4D(*Pl*)	*c-D4D(Pl)*	*Pavlova lutheri*
Acetohydroxy acid synthase	AHAS(*At*)	*c-AHAS(At)*	*Arabidopsis thaliana*

Fatty acid elongation, extending existing C18 fatty acids by C2 units, requires a four-step reaction cycle that includes ketoacyl synthase (KS), ketoacyl reductase, hydroxyacyl dehydratase and enoyl reductase activities analogous to the corresponding reaction cycle in de novo fatty acid synthesis (Haslam & Kunst, 2013; Jakobsson et al., 2006; Leonard et al., 2004). In contrast to de novo fatty acid synthesis, the elongation enzymes are membrane bound, localised in the endoplasmic reticulum and the acyl chain substrate is bound to coenzyme A (CoA) instead of ACP (acyl carrier protein necessary to transfer the growing fatty acid during biosynthesis between reaction centres; Leonard et al., 2004).

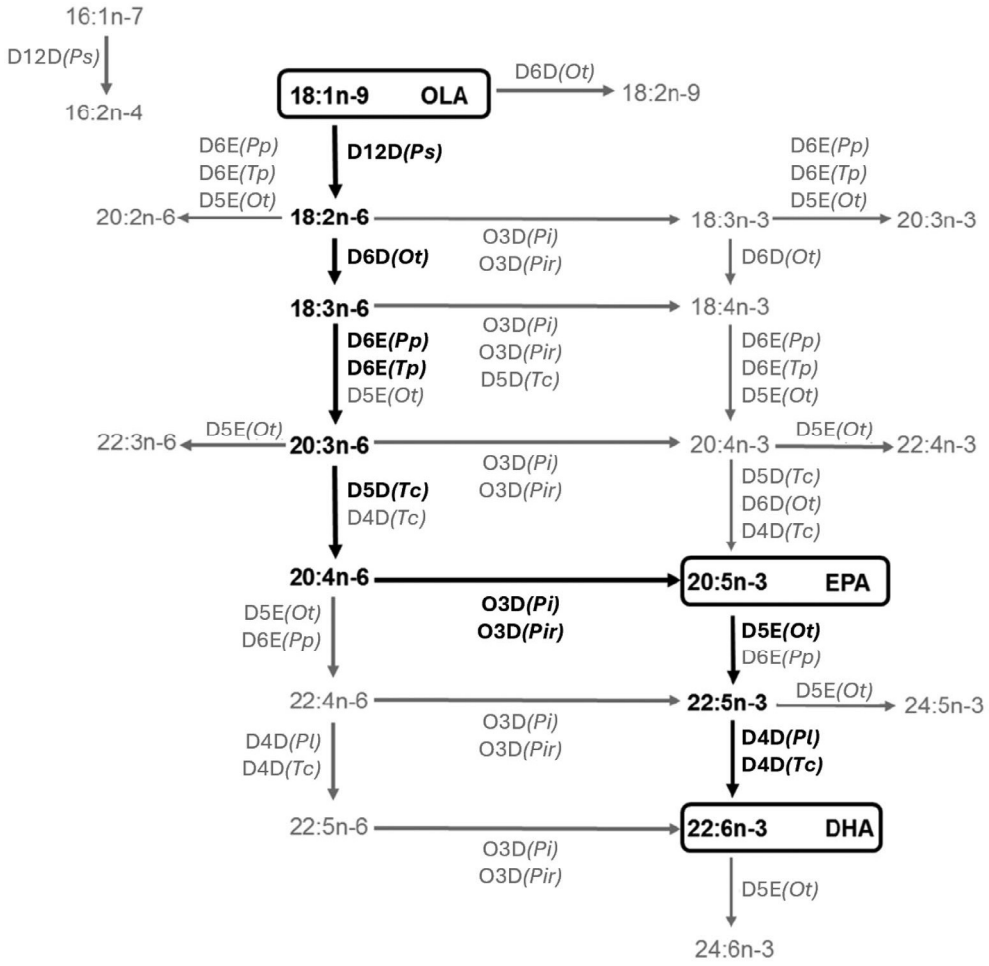

FIGURE 1 Depiction of putative metabolic network mediated by introduced NEPs as based on the substrate specificities of individual elongases and desaturases determined using in vivo feeding experiments in yeast. The originally anticipated linear metabolic pathway is shown in black, additional reactions proposed to take place in the yeast and approximated to oilseed rape LBFLFK seeds are in grey. Adapted from Yilmaz et al. (2017).

The term elongase is context-dependent and can refer to the entire complex including all four activities or just the KS component that catalyses the condensation of a C2 donor to an existing acyl chain acceptor. Similarly, to the fatty acid synthase complex (Cui et al., 2016; Harwood, 2005), the KS component of the elongation system was shown to be critical for determining substrate specificity (Denic & Weissman, 2007). The enzymes catalysing the remaining three steps of the elongation cycle appear to have a broad substrate tolerance, which was demonstrated in both yeast and plants (Millar & Kunst, 1997).

All of the elongase proteins newly expressed in oilseed rape LBFLFK display the structural features of an ELO-type KS: they are predicted to have five to seven transmembrane-spanning helices consistent with the currently accepted topology model for ELO-type KSs and possess the four motifs (HXXHH, KXX(E/D)XXDT, HXXMYXYY and TXXQXXQ) conserved among ELO-type KSs. The three newly expressed elongases were each demonstrated to catalyse the transfer of C2 units from malonyl-CoA to their respective acyl-CoA substrate. Additional evidence that all the newly expressed elongases are ELO-type KSs is found in the fact that these specific proteins are insensitive to an inhibitor (cerulenin) that inhibits all other KS protein superfamilies (Yilmaz et al., 2017).

Fatty acid desaturases catalyse the abstraction of two hydrogen atoms from the hydrocarbon chain of a fatty acid to form a double bond in an unsaturated fatty acid. Delta desaturases create the double bond at a fixed position from the carboxyl end of a fatty acid chain. For example, delta-9-desaturase creates a double bond between the ninth and tenth carbon atom from the carboxyl end. Compared to that, omega desaturases create the double bond at a fixed position from the methyl end of a fatty acid chain. For instance, omega-3-desaturase creates a double bond between the third and fourth carbon atom from the methyl end, thus creating an omega-3 fatty acid. Plants possess three different families of desaturases: soluble acyl-ACP desaturases in the stroma of plastids, prokaryotic type integral membrane acyl-lipid desaturases in the chloroplast membrane and eukaryotic type integral membrane acyl-lipid desaturases in the endoplasmic reticulum.

All the desaturases introduced into oilseed rape LBFLFK are predicted to have transmembrane-spanning helices consistent with the current accepted topology model for integral membrane desaturases and possess the three histidine boxes conserved among all integral membrane desaturases. All four newly expressed front-end desaturases D6D(*Ot*), D5D(*Tc*), D4D(*Tc*) and D4D(*Pl*) contain an N-terminal cytochrome b5 domain as well as the conserved glutamine in histidine box 3 while all three newly expressed methyl end desaturases D12D(*Ps*), O3D(*Pir*) and O3D(*Pi*) lack a fused N-terminal cytochrome b5 domain and have the expected histidine instead of glutamine in histidine box 3.

Each of these seven desaturases was shown to abstract two hydrogen atoms from the hydrocarbon chain of their respective fatty acid substrate, forming the expected unsaturated fatty acid product (Yilmaz et al., 2017).

3.2 | Systematic literature review

The GMO Panel assessed the applicant's literature searches on oilseed rape LBFLFK, which include a scoping review, according to the guidelines given in EFSA (2010, 2017b).

A systematic review as referred to in Regulation (EU) No 503/2013[3] has not been provided in support to the risk assessment of application EFSA-GMO-DE-2019-157. 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 LBFLFK at present.

The GMO Panel considered the overall quality of the literature searches performed acceptable. The literature searches identified four relevant publications on oilseed rape LBFLFK, listed in Appendix B, which were considered in this opinion as part of the risk assessment of oilseed rape LBFLFK.

3.3 | Molecular characterisation[6]

3.3.1 | Transformation process and vector constructs

Oilseed rape LBFLFK was developed by *Agrobacterium rhizogenes*-mediated transformation. Hypocotyl segments of 5-day-old Kumily seedlings were co-cultured with a disarmed *A. rhizogenes* strain SHA001 containing the vector LTM593. The plasmid LTM593 used for the transformation contains 13 expression cassettes (coding for 11 unique proteins, *c-D5D(Tc)* and *c-O3D(Pir)* included twice, see below) between the right and left border of the T-DNA, containing the following genetic elements:

- The *c-D12D*(*Ps*) expression cassette consists of the p-*napA*(*Bn*) seed-specific promoter from *Brassica napus*, the intron-containing 5′UTR (untranslated region) from the *Arabidopsis thaliana* locus At5g63190, the *B. napus* codon-optimised sequence of the *cD12D(Ps)* gene from *Phytophthora sojae* and the t-*rbcS*(*Ps*) small subunit of ribulose bisphosphate carboxylase protein terminator region from *Pisum sativum*.
- The *c-D6D*(*Ot*) expression cassette consists of the p-*SBP(Vf)* seed-specific promoter from *Vicia faba*, the intron-containing 5′UTR from the *A. thaliana* locus At1g65090, the *B. napus* codon-optimised sequence of the *cD6D(Ot)* gene from *Ostreococcus tauri* and the t-*CATHD*(*St*) cathepsin D inhibitor terminator region from *Solanum tuberosum*.
- The c-*D6E*(*Tp*) expression cassette consists of the p-*PXR*(*Lu*) seed-specific promoter from *Linum usitatissimum*, the intron-containing 5′UTR from the *A. thaliana* locus At1g62290, the *B. napus* codon-optimised sequence of the *cD6E(Tp)* gene from *Thalassiosira pseudonana* and the t-*PXR*(*At*) peroxiredoxin-like protein gene *PER1* terminator region from *A. thaliana*.
- The *c-D6E*(*Pp*) expression cassette consists of the p-*USP*(*Vf*) seed-specific promoter from *V. faba*, the intron-containing 5′UTR from the *A. thaliana* locus At1g01170, the *B. napus* codon-optimised sequence of the *cD6E(Pp)* gene from *Physcomitrella patens* and the *t-CaMV35S* 35S 3′UTR terminator region from Cauliflower Mosaic Virus.
- The *c-D5D*(*Tc*) (cassette 1) expression cassette consists of the p-*CNL*(*Lu*) seed-specific promoter from *L. usitatissimum*, the intron-containing 5′UTR from the *A. thaliana* locus At5g63190, the *B. napus* codon-optimised sequence of the *cD5D(Tc)* gene from *Thraustochytrium* sp. and the t-*OCS* octopine synthase gene terminator region from *A. tumefaciens* plasmid pTi15955.
- The *c-D5D*(*Tc*) (cassette 2) expression cassette consists of the p-*SETL*(*Bn*) seed-specific promoter from *B. napus*, the *B. napus* codon-optimised sequence of the *cD5D(Tc)* gene from *Thraustochytrium* sp. and the t-*SETL*(*Bn*) *SETL* gene terminator region from *B. napus*.
- The *c-O3D*(*Pi*) expression cassette consists of the p-*USP*(*Vf*) seed-specific promoter from *V. faba*, the intron-containing 5′UTR from the *A. thaliana* locus At1g01170, the *B. napus* codon-optimised sequence of the *c-O3D*(*Pi*) gene from *Phytophthora infestans* and the t-*CaMV35S* 3′UTR terminator region from Cauliflower Mosaic Virus.
- The *c-O3D*(*Pir*) (cassette 1) expression cassette consists of the p-*SETL*(*Bn*) seed-specific promoter from *B. napus*, the *B. napus* codon-optimised sequence of the *c-O3D*(*Pir*) gene from *Pythium irregulare* and the t-*SETL*(*Bn*) *SETL* gene terminator region from *B. napus*.
- The *c-O3D*(*Pir*) (cassette 2) expression cassette consists of the p-*PXR*(*Lu*) seed-specific promoter from *L. usitatissimum*, the intron-containing 5′UTR intron from the *A. thaliana* gene *AGO4*(*At*), the *B. napus* codon-optimised sequence of the *cO3D(Pir)* gene from *Pythium irregulare* and the t-*PXR*(*At*) 3′UTR peroxiredoxin-like protein gene PER1 terminator region from *A. thaliana*.
- The *c-D5E*(*Ot*) expression cassette consists of the p-*FAE1*(*Bn*) seed-specific promoter from *B. napus*, the intron-containing 5′UTR from the *A. thaliana* locus At1g62290, the *B. napus* codon-optimised sequence of the *cD5E(Ot)* gene from *O. tauri* and the t-*FAE1*(*At*) 3′UTR of a fatty acid elongase gene terminator region from *A. thaliana*.

[6]Dossier: Part II – Section 1.2; additional information: 20/5/2020, 8/12/2020, 31/3/2021, 24/9/2021, 30/9/2022, 17/7/2023 and 3/4/2024.

- The *c-D4D*(*Tc*) expression cassette consists of the p-*ARC5*(*Pv*) seed-specific promoter from *Phaseolus vulgaris*, the *B. napus* codon-optimised sequence of the *cD4D(Tc)* gene from *Thraustochytrium* sp. and the t-*ARC(Pv)*, the *Arc5* gene 3′UTR region from *P. vulgaris*.
- The *c-D4D*(*Pl*) expression cassette consists of the p-*CNL*(*Lu*) seed-specific promoter from *L. usitatissimum*, the intron-containing 5′UTR from the *A. thaliana* locus At1g65090, the *B. napus* codon-optimised sequence of the *c-D4D*(*Pl*) gene from *Pavlova lutheri* and the t-*OCS* octopine synthase gene terminator region from *A. tumefaciens* plasmid pTi15955.
- The *c-AHAS*(*At*) expression cassette consists of the p-*Ubi4*(*Pc*) ubiquitin promoter from *Petroselinum crispum*, the intron-containing 5′UTR from the *P. crispum* ubiquitin i-*Ubi4*(*Pc*), the coding sequence of the *c-AHAS*(*At*) gene from *A. thaliana* and the t-*AHAS*(*At*) AHAS large subunit gene polyadenylation and terminator region from *A. thaliana*.

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

3.3.2 | Transgene constructs in the GM plant

Molecular characterisation of oilseed rape LBFLFK was performed by next-generation sequencing (NGS) to determine insert copy number and to confirm the absence of plasmid backbone sequences, by polymerase chain reaction (PCR) followed by Sanger sequencing to determine the 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 with the requirements listed in the EFSA Technical Note (EFSA GMO Panel, 2018).

NGS data indicated that oilseed rape LBFLFK contains two inserts (Insert 1 and Insert 2), each consisting of a single copy of the T-DNA originating from the LTM593 transformation vector. NGS also indicated the absence of vector backbone sequences.

The nucleotide sequences of the entire Insert 1 (46,288 bp) and Insert 2 (45,968 bp) of oilseed rape LBFLFK were determined as well as 1179 bp 5′ flanking and 1291 bp 3′ flanking sequence for Insert 1, and 1180 bp 5′ flanking and 1009 bp 3′ flanking sequence for Insert 2. Compared to the sequence of T-DNA in LTM593, the Insert 1 sequence reveals 2 nucleotide changes (C to A transition at position 16,076 and 30,645, leading to a phenylalanine to leucine amino acid substitution [F83L] in the D12D(*Ps*) protein) while the Insert 2 sequence reveals 1 nucleotide change (G to T transition at position 35,841, resulting in an alanine to serine amino acid substitution [A102S] in the D4D(*Pl*) protein). In addition, Insert 1 sequence contains at the 5′ end a 64 bp long rearranged fragment consisting of short right border (RB)-derived repeats, and 184 bp and 72 bp truncations at RB and left border (LB), respectively; Insert 2 sequence contains 184 bp and 53 bp truncations at RB and LB, respectively, and 2 bp and 4 bp additions at 5′ and 3′ insert sites, respectively. Moreover, 8 bp were deleted from the oilseed rape host genome for the insertion of Insert 1, and 31 bp were deleted for the insertion of Insert 2.

The possible interruption of known endogenous oilseed rape genes by the insertion of both Insert 1 and Insert 2 in oilseed rape LBFLFK was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the inserts. The results of these analyses do not indicate the interruption of any known endogenous gene in oilseed rape LBFLFK.

The results of segregation (see Section 3.3.5) and bioinformatics analyses establish that both Insert 1 and Insert 2 are located in the nuclear genome and are segregating independently.

Bioinformatics analyses of the amino acid sequence of all the newly expressed proteins reveal no significant similarities to toxins and allergens. In addition, updated bioinformatic analyses of the newly created open reading frames (ORFs) within the Insert 1 and Insert 2 and spanning the junctions between the inserts and genomic DNA indicate that the expression of any ORF showing significant similarities to toxins or allergens in oilseed rape LBFLFK is highly unlikely.

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 LBFLFK, which consists of expression cassettes containing *B. napus* codon-optimised NEP coding sequences, except AHAS, originating from *A. thaliana*, to microbial DNA (DB 2023). The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.6.1.2.

Some of the bioinformatic analyses were conducted using databases released in 2023.[7]

3.3.3 | Protein characterisation[8]

Brassica napus LBFLFK expresses 11 new proteins: D12D(*Ps*), D6D(*Ot*), D6E(*Tp*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D5E(*Ot*), D4D(*Pl*), D4D(*Tc*) and AHAS(*At*).

c-D5D(Tc) and *c-O3D(Pir)* are expressed twice, by two different expression cassettes each (see Section 3.3.1), while the coding regions of inserted D12D(*Ps*) and D4D(*Pl*) genes present, respectively, one nucleotide change compared to the T-DNA

[7]Dossier: Part II – Section 1.2.2.2; additional information: 30/9/2022, 3/4/2024.

[8]Dossier: Part II – section 1.4.1 and Study ID: 120051.

sequence of LTM593, resulting in one amino acid change for each protein (see Section 3.3.2). The expression of the introduced desaturases and elongases, combined with the *B. napus* native fatty acid synthesis enzymes, allows for the synthesis of long-chain polyunsaturated fatty acids (LC-PUFAs), including EPA and DHA, in the seeds of oilseed rape LBFLFK (see Section 3.1).

Prior to safety studies, a series of biochemical methods were employed to characterise these 11 newly expressed proteins in terms of their physicochemical, structural and functional properties.

As most of the NEPs could not be produced in a heterologous expression system in the amounts and purity sufficient for the protein characterisation, probably because they are membrane-spanning proteins, a membrane fraction purified from crude extracts of developing embryos was isolated from embryos was isolated from immature seeds of oilseed rape LBFLFK and used for characterisation. This detergent-free membrane fraction, referred to herein as 'plant-produced proteins' (PPP), contains active and full-length elongases and desaturases. Newly expressed proteins [D12D(*Ps*), D6D(*Ot*), D6E(*Pp*), D6E(*Tp*), D5D(*Tc*), O3D(*Pi*), O3D(*Pir*), D5E(*Ot*), D4D(*Pl*), D4D(*Tc*)] and AHAS(*At*) [A122TS653N] in event LBFLFK that are present in the PPP fractions were characterised for their (i) apparent molecular weights and immunoreactivity by western blotting, (ii) identity by peptide mapping using mass spectrometry, (iii) glycosylation status by selective carbohydrate biotinylation and western blotting, (iv) enzyme activity by an in vitro assay, and (v) quantity by validated quantitative assay. For the immunodetection of the PPP, specific antibodies were developed using either expression and purification of NC-fusion of the corresponding NEP (the fusion of N- and C-terminal ends of the protein, missing the central part facilitating the expression in bacterial expression system) or synthesis of peptides selected from the amino acid sequence of the corresponding NEP. As a negative control, the membrane fraction from the immature seeds (embryos) of the parental oilseed rape variety Kumily (not containing the NEPs) was used.

Membrane fractions from yeast strains producing the D6D or D5D proteins encoded by the *D6D(Ot) or D5D(Tc)* genes, respectively, were prepared and served as positive controls for the respective in vitro enzyme activity assays in the study No.2 01601-008.[9]

The substrate specificity of the three elongases and seven desaturases introduced into oilseed rape LBFLFK was assessed using yeast strains individually expressing each of the proteins (Yilmaz et al., 2017). In this publication, in vivo feeding studies were performed in these yeast strains with 14 potential fatty acid intermediates in the fatty acid pathway to allow an assessment of specificity of each expressed enzyme. Additionally, using membranes isolated from these yeast expression strains, in vitro assays were used to assess the backbone specificity of each of the elongases and desaturases.

The in vivo feeding studies demonstrated that each desaturase and elongase is capable of acting on multiple fatty acid substrates. In the context of the conversion of OLA to DHA, this means that each enzyme can catalyse multiple reactions in the pathway, and that each reaction may be catalysed by multiple enzymes. Therefore, when the 10 enzymes are combined in plant seed, one might expect DHA to be synthesised via a network rather than a linear series of reactions. The ability to model such a network will provide a more complete understanding of the fatty acid profile obtained when combining these 10 enzymes into a single cell or organism (Yilmaz et al., 2017).

D12D(Ps) characterisation. Western blot analysis showed that D12D(*Ps*) had the expected molecular weight of ~41.8 kDa and was immunoreactive to D12D(Ps) protein-specific antibodies. In addition, glycosylation detection analysis suggested that a fraction of the D12D(Ps) protein might be glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D12D(Ps) amino acid sequence matched the deduced sequence as defined by the *D12D(Ps)* coding sequence. Enzymatic activity was demonstrated by a biochemical in vitro activity assay which showed that D12D(Ps) had the expected desaturase activity for the intended substrate.

D12D(Ps) conclusions. The data confirm the presence of D12D(*Ps*) in the PPP, exhibiting enzymatic activity for the intended substrate. Based on the data provided, the presence of partially glycosylated D12D(*Ps*) in the PPP could not be excluded.

D6D(Ot) characterisation. Western blot analysis showed that D6D(*Ot*) had the expected molecular weight of ~55.6 kDa and was immunoreactive to D6D(*Ot*) protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that a fraction of the D6D(*Ot*) protein might be glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D6D(*Ot*) amino acid sequence matched the deduced sequence as defined by the D6D*(Ot)* coding sequence. In contrast to a membrane fraction obtained from a yeast strain expressing the D6D(*Ot*) protein revealing anticipated activity, the D6D(*Ot*) enzymatic activity assessed by a biochemical in vitro activity assay was undetectable in the PPP for the intended substrate.

D6D(Ot) conclusions. The data confirm the presence of D6D(*Ot*) in the PPP. Based on the data provided, the presence of partially glycosylated D6D(*Ot*) in the PPP could not be excluded and its enzymatic activity could not be detected.

D6E(Tp) characterisation. Western blot analysis showed that D6E(*Tp*) had the expected molecular weight of ~22 kDa and was immunoreactive to D6E(*Tp*) protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that D6E(*Tp*) was not glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D6E(*Tp*) amino acid sequence matched the deduced sequence as defined by the *D6E(Tp)* coding sequence. Enzymatic activity was demonstrated by a biochemical in vitro activity assay which showed that D6E(*Tp*) had the expected desaturase activity for the intended substrate. However, the activity assay could not distinguish between the individual D-6-elongases because of the similarity in substrate specificity (overlapping). Since the amount of D6E(*Pp*) was below the limit of detection (LOD), it is assumed that the detected D6E activity is fully attributed to D6E(*Tp*).

D6E(Tp) conclusions. The data confirm presence of non-glycosylated D6E(*Tp*) in the PPP exhibiting enzymatic activity for the intended substrate.

[9]Dossier: Part II – Section 1.2; additional information: 11/10/2022.

D6E(Pp) characterisation. No peptides specific for the D6E(*Pp*) protein were observed using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Accordingly, western blot analysis showed that D6E(*Pp*) could not be detected by D6E(*Pp*) protein-specific antibodies. In addition, given the absence of signal in the western blot analysis, glycosylation detection analysis could not be performed as the assay was based on the use of D6E(*Pp*) protein-specific antibodies. Amino acid sequence analysis by mass spectrometry showed that no peptides specific to the D6E(*Pp*) protein could be detected.

D6E(Pp) conclusions. The presence of D6E(*Pp*), its glycosylation status and enzymatic activity for the intended substrate in the PPP were not demonstrated.

D5D(Tc) characterisation. Western blot analysis showed that D5D(*Tc*) had the expected molecular weight of ~49.8 kDa and was immunoreactive to D5D(*Tc*) protein-specific antibodies. In addition, glycosylation detection analysis suggested that a fraction of the D5D(*Tc*) protein might be glycosylated. Amino acid sequence analysis by mass spectrometry confirmed the identity of the D5D(*Tc*) as defined by the *D5D(Tc)* coding sequence. D5D(*Tc*) enzymatic activity assessed by a biochemical in vitro activity assay was undetectable in the PPP for the intended substrate, compared to a membrane fraction obtained from a yeast strain expressing the D5D(*Tc*) protein which showed observable activity.

D5D(Tc) conclusions. The data confirm presence of D5D(*Tc*) in the PPP, but its *in planta* activity was not detectable. Based on the data provided, the presence of partially glycosylated D5D(*Tc*) in the PPP could not be excluded.

O3D(Pir) characterisation. Western blot analysis showed that O3D(*Pir*) had the expected molecular weight of ~40.4 kDa and was immunoreactive to O3D(*Pir*) protein-specific antibodies. In addition, glycosylation detection analysis suggested that a fraction of O3D(*Pir*) protein might be glycosylated. Amino acid sequence analysis showed that the O3D(*Pir*) amino acid sequence matched the deduced sequence as defined by the *O3D(Pir)* coding sequence. Nonetheless, O3D(*Pir*) enzymatic activity assessed by a biochemical in vitro activity assay was undetectable for the intended substrate.

O3D(Pir) conclusions. The data confirm presence of O3D(*Pir*) in the PPP. Based on the data provided the presence of partially glycosylated O3D(*Pir*) in the PPP could not be excluded and its enzymatic activity could not be detected.

O3D(Pi) characterisation. Western blot analysis showed that O3D(*Pi*) could not be detected by O3D(*Pi*) protein-specific antibodies. In addition, given the absence of signal in the western blot analysis, glycosylation detection analysis could not be performed as the assay was based on the use of O3D(*Pi*) protein-specific antibodies. Amino acid sequence analysis by mass spectrometry showed that no peptides specific to the O3D(*Pi*) protein could be detected. O3D(*Pi*) enzymatic activity assessed by a biochemical in vitro activity assay was undetectable for the intended substrate.

O3D(Pi) conclusions. The presence of O3D(*Pi*) in the PPP, its glycosylation status and in planta activity were not demonstrated.

D5E(*Ot*) *characterisation*. Western blot analysis showed that D5E(*Ot*) had the expected molecular weight of ~34.2 kDa and was immunoreactive to D5E(*Ot*) protein-specific antibodies. The glycosylation detection analysis demonstrated that a fraction of the D5D(*Tc*) might be glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D5E(*Ot*) amino acid sequence matched the deduced sequence as defined by the D5E(*Ot*) coding sequence. Enzymatic activity was demonstrated by a biochemical in vitro activity assay which showed that D5E(*Ot*) had the expected elongase activity for the intended substrate.

D5E(Ot) conclusions. The data confirm the presence of D5E(*Ot*) in the PPP revealing enzymatic activity for the intended substrate. Based on the data provided, the presence of partially glycosylated D5E(*Ot*) in the PPP could not be excluded.

D4D(*Pl*) *characterisation*. Western blot analysis showed that D4D(*Pl*) had the expected molecular weight of ~49.1 kDa and was immunoreactive to D4D(*Pl*) protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that a fraction of the D4D(*Pl*) might be glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D4D(*Pl*) amino acid sequence matched the deduced sequence as defined by the *D4D*(*Pl*) coding sequence. D4D enzymatic activity for the intended substrate was demonstrated by a biochemical in vitro activity assay. However, because of the overlapping substrate specificities, the activity assay could not distinguish between the D4D(*Pl*) and D4D(*Tc*) individual D4-desaturases.

D4D(*Pl*) *conclusions*. The data confirm presence of D4D(*Pl*) in the PPP. Based on the data provided the presence of partially glycosylated D4D(*Pl*) in the PPP could not be excluded and the D4D(*Pl*) *in planta* activity was not unambiguously proven.

D4D(*Tc*) *characterisation*. Western blot analysis showed that D4D(*Tc*) had a molecular weight of ~63 kDa close to the expected molecular weight of ~59 kDa and was immunoreactive to D4D(*Tc*) protein-specific antibodies. The glycosylation detection analysis suggested that a fraction of D4D(*Tc*) protein might be glycosylated. Amino acid sequence analysis by mass spectrometry showed that the D4D(*Tc*) amino acid sequence matched the deduced sequence as defined by the D4D *(Tc)* gene. D4D enzymatic activity for the intended substrate was demonstrated by a biochemical in vitro activity assay. However, because of the overlapping substrate specificities, the activity assay could not distinguish between the D4D(*Pl*) and D4D(*Tc*) desaturases.

D4D*(Tc) conclusions*. The data confirm presence of D4D(*Tc*) in the PPP. Based on the data provided the presence of partially glycosylated D4D(*Tc*) in the PPP could not be excluded and the D4D(*Tc*) *in planta* activity was not unambiguously proven.

AHAS(At) characterisation. Western blot analysis estimated that AHAS(*At*) migrated at 79.2 kDa, compared to the expected ~66.1 kDa and was immunoreactive to AHAS(*At*) protein-specific antibodies. In addition, glycosylation detection analysis demonstrated that AHAS(*At*) was not glycosylated. Amino acid sequence analysis by mass spectrometry showed that the AHAS(*At*) amino acid sequence matched the deduced sequence as defined by the *AHAS(At)* coding sequence. Herbicide (imazamox)-tolerant enzymatic AHAS activity for the intended substrate was demonstrated by a biochemical in vitro activity assay.

AHAS(At) conclusions. The data confirm presence of non-glycosylated AHAS(*At*) in the PPP exhibiting herbicide-tolerant activity for the intended substrate.

3.3.4 | Information on the expression of the inserts

Protein levels of D12D(*Ps*), D6D(*Ot*), D6E(*Tp*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D5E(*Ot*), D4D(*Tc*), D4D(*Pl*) and AHAS(*At*) were analysed by enzyme-linked immunosorbent assay (ELISA) and western blotting in material harvested in a field trial across four locations in the USA during the 2016 growing season. Samples analysed included immature seeds (BBCH 75–79) and mature seeds (BBCH 99) from plants treated and not treated with imazamox, an imidazolinone herbicide. The mean values, standard deviations and ranges of protein expression levels in mature and immature seeds ($n = 20$) of D12D(*Ps*), D6D(*Ot*), D6E(*Tp*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D5E(*Ot*), D4D(*Tc*), D4D(*Pl*), AHAS(*At*) proteins are reported in Table 2. The methodology used to quantify the levels of the proteins is considered adequate.

TABLE 2 Mean values, standard deviations and ranges of newly expressed proteins in immature and mature seeds [μg/g dry weight (dw) and μg/g fresh weight (fw)] from oilseed rape LBFLFK ($n = 20$).

	Imazamox treatment			
	Not treated		Treated	
Tissues	μg/g dry weight (dw)	μg/g fresh weight (fw)	μg/g dry weight (dw)	μg/g fresh weight (fw)
Immature seed (BBCH 75–79)				
D12D(*Ps*)	6.50[a] ± 4.26[b] (< LOD[e]–15.63)[c]	1.56 ± 0.65 (< LOD-3.18)[e]	4.76 ± 3.59 (< LOD-12.06)[e]	1.25 ± 0.61 (< LOD-2.61)[e]
D6D(*Ot*)	35.15 ± 28.26 (< LOD-96.51)[e]	10.50 ± 9.44 (< LOD-38.76)[e]	28.00 ± 24.68 (< LOD-80.39)[e]	7.76 ± 6.27 (< LOD-20.88)[e]
D6E(*Tp*)	829.94 ± 271.32 (< LOQ-1452.54)[d]	219.88 ± 62.21 (< LOQ-370.48)[d]	826.70 ± 261.32 (< LOQ-1120)[d]	210.77 ± 47.75 (< LOQ-281.32)[d]
D6E(*Pp*)	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]
D5D(*Tc*)	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]
O3D(*Pir*)	121.82 ± 52.73 (< LOD-243.60)[e]	37.93 ± 17.03 (< LOD-97.83)[e]	90.27 ± 41.44 (< LOD-209.12)[e]	29.61 ± 10.80 (< LOD-56.98)[e]
O3D(*Pi*)	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]
D5E(*Ot*)	< LOQ[d,g] (< LOD-28.39)[e]	< LOQ[d,g] (< LOD-11.4)[e]	– (< LOD-16.24)[e]	– (< LOD-4.55)[e]
D4D(*Tc*)	– (< LOD–< LOQ)[d,e]	– (< LOD–< LOQ)[d,e]	– (< LOD-22.73)[e]	– (< LOD-4.92)[e]
D4D(*Pl*)	< LOQ[d,g] (< LOD-9.29)[e]	< LOQ[d,g] (< LOD-2.34)[e]	< LOQ[d,g] (< LOD-12.20)[e]	< LOQ[d,g] (< LOD-3.17)[e]
AHAS(*At*)	11.71 ± 4.04 (< LOQ-20.33)[d]	3.12 ± 0.76 (< LOQ-4.62)[d]	11.96 ± 3.78 (< LOQ-17.00)[d]	2.95 ± 0.67 (< LOQ-4.25)[d]
Mature seed (BBCH 99)				
D12D(*Ps*)	0.83 ± 0.29 (< LOQ-1.42)[d]	0.74 ± 0.23 (< LOQ-1.20)[d]	0.80 ± 0.27 (< LOQ-1.50)[d]	0.73 ± 0.26 (< LOQ-1.40)[d]
D6D(*Ot*)	32.24 ± 14.50 (15.27–64.80)	29.02 ± 13.26 (14.41–62.31)	31.20 ± 10.65 (18.53–51.56)	28.80 ± 10.28 (17.65–49.58)
D6E(*Tp*)	679.42 ± 132.94 (409.98–939.00)	610.21 ± 91.49 (383.16–739.98)	682.75 ± 100.10 (546.23–913.16)	628.99 ± 94.85 (514.25–878.04)
D6E(*Pp*)	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]
D5D(*Tc*)	< LOQ[d,g] (< LOD-1.37)	< LOQ[d,g] (< LOD-1.32)	< LOQ[d,g] (< LOD-2.04)	< LOQ[d,g] (< LOD-1.98)
O3D(*Pir*)	171.53 ± 58.53 (< LOQ-303.67)[d]	151.57 ± 42.30 (< LOQ-233.59)[d]	172.56 ± 51.18 (< LOQ-288.98)[d]	156.43 ± 40.85 (< LOQ-233.05)[d]
O3D(*Pi*)	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]	< LOD[e,f]
D5E(*Ot*)	7.37 ± 3.18 (< LOQ-14.60)[d]	6.96 ± 3.12 (< LOQ-14.04)[d]	8.15 ± 2.89 (< LOQ-13.79)[d]	7.65 ± 2.83 (< LOQ-13.13)[d]
D4D(*Tc*)	– (< LOD- < LOQ)[d,e]	– (< LOD- < LOQ)[d,e]	– (< LOD-10.30)[e]	– (< LOD-10)[e]
D4D(*Pl*)	< LOQ[d,g] (< LOD-6.90)[e]	< LOQ[d,g] (< LOD-6.57)[e]	< LOQ[d,g] (< LOD-8.78)[e]	< LOQ[d,g] (< LOD-8.21)[e]
AHAS(*At*)	3.27 ± 0.40 (< LOQ-4.14)[d]	3.03 ± 0.43 (< LOQ-3.98)[d]	3.43 ± 0.57 (< LOQ-5.17)[d]	3.20 ± 0.59 (< LOQ-4.92)[d]

Abbreviation: –, Not applicable (data sets did not contain two or more quantifiable values).

[a]Mean value.

[b]Standard deviation.

[c]Range.

[d]< LOQ = below the limit of quantitation; LOQ for D12D(*Ps*) = 0.55 μg/g dw and 0.51 μg/g fw; LOQ for D6E(*Tp*) = 448.51 μg/g dw and 153.60 μg/g fw; LOQ for D5D(*Tc*) = 1.31 μg/g dw and 1.20 μg/g fw; LOQ for O3D(*Pir*) = 117.60 μg/g dw and 107.89 μg/g fw; LOQ for D5E(*Ot*) = 13.03 μg/g dw and 4.46 μg/g fw in immature seed and 4.87 μg/g dw and 4.46 μg/g fw in mature seed; LOQ for D4D(*Pl*) = 5.63 μg/g dw and 1.93 μg/g fw in immature seed and 4.21 μg/g dw and 3.86 μg/g fw in mature seed; LOQ for D4D(*Tc*) = 8.83 μg/g dw and 3.02 μg/g fw in immature seed and 9.23 μg/g dw and 8.47 μg/g fw in mature seed; LOQ for AHAS(*At*) = 6.12 μg/g dw and 2.10 μg/g fw in immature seed and 3.05 μg/g dw and 2.80 μg/g fw in mature seed.

[e]< LOD = below the limit of detection; LOD for D12D(*Ps*) = 0.70 μg/g dw and 0.24 μg/g fw; LOD for D6D(*Ot*) = 5.26 μg/g dw and 1.80 μg/g fw; LOD for D6E(*Pp*) = 2.78 μg/g dw and 0.95 μg/g fw in immature seed and 1.04 μg/g dw and 0.95 μg/g fw in mature seed; LOD for D5D(*Tc*) = 11.21 μg/g dw and 3.84 μg/g fw in immature seed and 0.52 μg/g dw and 0.48 μg/g fw in mature seed; LOD for O3D(*Pir*) = 33.37 μg/g dw and 11.43 μg/g fw; LOD for O3D(*Pi*) = 7.26 μg/g dw and 2.48 μg/g fw in immature seed and 27.08 μg/g dw and 24.85 μg/g fw in mature seed; LOD for D5E(*Ot*) = 2.84 μg/g dw and 0.97 μg/g fw; LOD for D4D(*Pl*) = 2.68 μg/g dw and 0.92 μg/g fw in immature seed and 2.00 μg/g dw and 1.83 μg/g fw in mature seed; LOD for D4D(*Tc*) = 4.84 μg/g dw and 1.66 μg/g fw in immature seed and 3.80 μg/g dw and 3.49 μg/g fw in mature seed.

[f]All samples were below LOD.

[g]Mean value fell below the limit of quantitation once < LOD and < LOQ results were substituted for their respective LOD and LOQ values.

3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of oilseed rape LBFLFK Inserts 1 and 2 was assessed using NGS to sequence the inserts and the flanking regions from five consecutive generations (T3, T4, T5, T6 and T7). The results indicate that all the plants tested retained the two inserts and flanking regions, which were stable in subsequent generations. Segregation analysis was performed by PCR-based analysis from three consecutive generations (F2, F3, F4). The results of the segregation analysis support the presence of two inserts (Insert 1 and Insert 2), independently segregating in a Mendelian fashion.

Phenotypic stability was assessed by determining the expression of the NEPs using two methods: (i) determination of the level of expression of the NEPs in two generations of self-pollination (T3 and T5); (ii) analysis of the fatty acid composition of the seed and determination of the imidazolinone tolerance trait by observing the herbicide damage of imazamox-treated plants in four generations of self-pollination (T4, T5, T6 and T7). The fatty acid composition analysis showed the increase in EPA and DHA and the concomitant decrease in oleic acid, while the herbicide tolerance test showed the absence of observed herbicide damage in oilseed rape LBFLFK plants sprayed with imazamox as compared to the conventional counterpart Kumily.

3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that oilseed rape LBFLFK contains two inserts (Insert 1 and Insert 2), not genetically linked, each consisting of a single copy of the T-DNA originating from the same transformation vector, which contains 13 expression cassettes (coding for 11 unique proteins). Bioinformatics analyses of the sequences encoding the newly expressed proteins and other ORFs within the inserts or spanning the junctions between the inserts and genomic DNA do not raise any safety concerns. The stability of the inserted DNA and of the introduced traits conferring herbicide tolerance and an altered fatty acid composition was confirmed over several generations. As most of the NEPs could not be produced in a heterologous expression system in the amounts and purity sufficient for the protein characterisation, the description of biochemical, structural and functional properties of the NEPs was performed with a membrane fraction purified from crude extracts of developing embryos that were isolated from immature seeds of oilseed rape LBFLFK (PPP). The activities of all the introduced elongases and desaturases for the intended substrates were demonstrated upon expression in yeast. However, several protein characterisation assays failed for a number of NEPs as PPP. These include protein detection using LC–MS/MS and/or western blotting that was not successful for D6E(*Pp*) and O3D(*Pi*), and absence of activity detection specific for D6D(*Ot*), D6E(*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D4D(*Pl*) and D4D(*Tc*). Moreover, based on the provided data, partial glycosylation of D12D(*Ps*), D6D(*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), D5E(*Ot*) and D4D(*Tc*) cannot be excluded. Due to failure of some of the analyses, the GMO panel cannot conclude that a membrane fraction purified from crude extracts of developing embryos is an appropriate source of NEP for the safety studies.

3.4 | Comparative analysis[10]

3.4.1 | Overview of studies conducted for the comparative analysis

Application EFSA-GMO-DE-2019-157 presents data on agronomic and phenotypic characteristics, as well as seed composition of oilseed rape LBFLFK (Table 3). In addition, the application contains data on seed dormancy from oilseed rape LBFLFK (see Table 3 and Appendix A).

TABLE 3 Main comparative analysis studies to characterise oilseed rape LBFLFK provided in the application EFSA-GMO-DE-2019-157.

Study focus	Study details	Comparator	Non-GM reference varieties
Agronomic, phenotypic analysis	Field study, USA, 2016, 13 sites[a]	Kumily	6[b]
Compositional analysis	Field study, USA, 2016, 12 sites[a]		

Abbreviation: GM, genetically modified.

[a]The field trials were located in Ephrata, WA; Idaho Falls, ID; Bozeman, Vaughn and Power MT; Northwood, ND (two fields); Brookings, SD (two fields); Campbell and Sartell MN and Lime Springs, IA. A field trial established in Grand Island, NE was partially included in the agronomic and phenotypic statistical analysis because of excessive heat during the flowering period; the data collected after the exceptional weather event were excluded from the statistical analysis. A field trial in Malta, MT was excluded from the statistical analysis due to hail and heavy rain occurred at the start of the growing season.

[b]The oilseed rape non-GM reference lines used in the field trials are: 46A65, IMC105, IMC302, Wizzard, Orinoco and V3002.

[10]Dossier: Part II – Section 1.3; additional information: 4/2/2020 and 28/4/2021.

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 LBFLFK not exposed to the intended herbicide (not treated), oilseed rape LBFLFK exposed to the intended herbicide (treated), the comparator Kumily and six commercial non-GM oilseed rape 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 LBFLFK, the application of a difference test (between the GM oilseed rape and the non-GM -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).[11]

3.4.3 | Suitability of selected test materials

3.4.3.1 | *Selection of the test materials*

The conventional oilseed rape variety Kumily was transformed to obtain oilseed rape LBFLFK that after stabilisation[12] was used in the comparative studies reported in Table 3. The comparator used in the field trials is the variety Kumily, that is therefore considered to be the conventional counterpart.

Six commercial non-GM reference varieties were selected by the applicant that were tested at each site.

Before starting the comparative analysis, oilseed rape LBFLFK, the conventional counterpart and the non-GM reference varieties were cultivated by the applicant to collect major agronomic and compositional data including plant height, lodging, days to maturity, yield, grain moisture, grain size, grain crude fat and protein content. Based on the information provided, the GMO Panel considers the test material appropriate for the comparative assessment.

3.4.3.2 | *Seed production and quality*

The seeds of GM oilseed rape LBFLFK, the conventional counterpart and the non-GM reference varieties used in the field trials (see Table 3) were produced, harvested and stored under similar conditions. The seed lots were verified for their purity via event-specific quantitative polymerase chain reaction analysis. The seeds were tested for their germination capacities and the results indicate that the seed germination of oilseed rape LBFLFK under warm conditions is not different from that of the conventional counterpart (Kumily). The GMO Panel considers that the starting seed used as test material in the agronomic, phenotypic and compositional studies was of suitable quality.

3.4.3.3 | *Conclusion on suitability*

The GMO Panel is of the opinion that oilseed rape LBFLFK, its conventional counterpart and the selected non-GM oilseed rape reference varieties are of adequate quality. Therefore, the test materials are considered acceptable for the comparative analysis.

3.4.4 | Representativeness of the receiving environments

3.4.4.1 | *Selection of field trial sites*

The soil and climatic characteristics of the selected fields were diverse and located in the US, but outside the most important commercial oilseed rape-growing regions of North America. The selection of the field trial sites was made because the requirement of oilseed rape LBFLFK to be cultivated in isolation from conventional oilseed rape, using an identity preservation system. The GMO Panel considers that the selected sites reflect 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 sums of precipitation were provided for each site on a weekly basis. Some exceptional weather conditions were reported at five of the selected sites[13] and resulted in the loss of some data at

[11]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).

[12]As documented by the pedigree, the T_5 generation has been used.

[13]Hail storm resulting in plant defoliation and stress at Bozeman, MT; precipitation was above normal and hail resulting in slight damage to plots at the two field trials in Northwood, ND; temperatures were above normal during the pollination period at Grand Island, NE and precipitation in April, May and June was below normal at Sartell, MN.

site in Grand Island, NE. However, due to the lack of major impacts on plant growth at these sites, the GMO Panel considers that the exceptional weather conditions did not invalidate the selection of the field trial sites for the comparative analyses.

3.4.4.3 | *Management practices*

The field trials included plots containing oilseed rape LBFLFK, plots with the conventional counterpart and plots with non-GM oilseed rape reference varieties, mostly managed according to local agricultural practices. In addition, the field trials included plots containing oilseed rape LBFLFK managed following the same agricultural practices, and exposed to Imazamox, selected as a representative of intended imidazolinone-containing herbicides, and used at growth stage BBCH 13–15. The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were acceptable for the field trials.

3.4.4.4 | *Conclusion on representativeness*

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and most of the management practices of the field trial sites reflect oilseed rape-growing regions in which the test materials are likely to be grown.

3.4.5 | Agronomic and phenotypic analysis

Eighteen agronomic and phenotypic endpoints plus information on biotic and abiotic stressors were collected from the field trial sites (see Table 3).[14] Five endpoints measured on a discrete scale (seedling vigour, ground cover at BBCH 65, plant lodging, pod shattering and green seed) were excluded from the statistical analysis because of a high number of uniform values in the data set.

The statistical analysis (Section 3.4.2) was applied to 13 endpoints, with the following results:

- For oilseed rape LBFLFK (not treated with the intended herbicide), the test of difference identified statistically significant differences with the conventional counterpart for ground cover at BBCH 12–13, final plant stand, plot yield and days to maturity. All the endpoints fell under equivalence category I or II.
- For oilseed rape LBFLFK (treated with the intended herbicide), the test of difference identified statistically significant differences with the conventional counterpart for field emergence, ground cover at BBCH 12–13, days to maturity and plot yield. All the endpoints fell under equivalence category I or II.

In addition, the applicant generated data on the germination of seeds harvested from the field trials (F_2 generation) under standard, warm and cold conditions and at different time points. In the statistical analysis (Section 3.4.2), the germination of oilseed rape LBFLFK (both treated and not treated) was found significantly delayed and reduced with respect to the conventional counterpart and the non-GM reference varieties (equivalence category III or IV for almost all conditions and stages). These results are assessed for potential environmental impact in Section 3.6.1.

3.4.6 | Compositional analysis

Oilseed rape LBFLFK seeds harvested from 12 sites (Table 3) were analysed for 114 constituents, including those recommended by the Organisation for Economic Co-operation and Development (OECD, 2011). The list of constituents includes several fatty acids to assess the intended changes in the fatty acid profile of oilseed rape LBFLFK. The statistical analysis was not applied to 38 seed constituents[15] as they were considered unsuitable for ANOVA methods because of the large number of measurements below the limit of quantification (LOQ) and/or the very low variability of the data set.

[14]Early stand count, days to 50% pollen shed, days to 50% silking, total lodging, plant height, days to maturity, final stand count, total lodging, grain/seed moisture, grain yield/yield and 1000-kernel weight.

[15]Glucoiberin, glucoraphanin, gluconapoleiferin, neoglucobrassicin, tannins, sodium, copper, Δ-7 avenasterol, campestanol, Δ-5,23 stigmastadienol, clerosterol, sitostanol, D-5,24 stigmastadienol, myristic acid, hexadecenoic acid, C16:1 trans, hexadecatrienoic acid, heptadecanoic acid, heptadecenoic acid, C18:1 trans, octadecadienoic acid, C18:2 trans, γ-linolenic acid, stearidonic acid, ω9-eicosadienoic acid, eicosatrienoic acid, dihomo- γ-linolenic acid, mead acid, bishomostearidonic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosadienoic acid, docosatetraenoic acid, adrenic acid, clupanodonic acid, osbond acid, docosahexaenoic acid.

The statistical analysis was applied to a total of 76 constituents in seeds[16]; a summary of the outcome of the test of difference and the test of equivalence is presented in Table 4:

- For oilseed rape LBFLFK not treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 52 endpoints in seeds. All these endpoints fell under equivalence category I or II, except for calcium, C16:1ω7 (palmitoleic acid), C18:0 (stearic acid), OLA, C20:1ω9 (gondoic acid), C20:2ω6 (ω6-eicosadienoic acid) and total trans-fatty acids (TFAs) which fell under equivalence category IV.
- For oilseed rape LBFLFK treated with the intended herbicide, statistically significant differences with the conventional counterpart were found for 44 endpoints in seeds. All these endpoints fell under equivalence category I or II, except for calcium, palmitoleic acid, stearic acid, OLA, gondoic acid, ω6-eicosadienoic acid and TFA which fell under equivalence category IV.

TABLE 4 Outcome of the comparative compositional analysis in seeds for oilseed rape LBFLFK. The table shows the number of endpoints in each category.

		Test of difference[a]			
		Not treated[b]		Treated[b]	
		Not different	Significantly different	Not different	Significantly different
Test of equivalence[c]	Category I/II	24	45[d]	32	37[d]
	Category III/IV	–	7[e]	–	7[e]
	Not categorised	–	–	–	–
	Total endpoints	76		76	

[a]Comparison between oilseed rape LBFLFK and its conventional counterpart.

[b]Treated/not treated with the intended herbicide.

[c]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.

[d]Endpoints with significant differences between oilseed rape LBFLFK and its conventional counterpart falling in equivalence category I–II. **Not treated**: proximates (moisture, crude fat, protein, crude fibre), amino acids (threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, tyrosine, phenylalanine, lysine, arginine, tryptophan, aspartic acid), vitamins (α-tocopherol, phylloquinone), minerals (phosphorus, magnesium, iron, zinc), sterols (brassicasterol, cholesterol, campesterol, stigmasterol, Δ-5 avenaterol, total phytosterols, 24-methylene cholesterol, Δ-7 stigmastenol), fatty acids (α-linolenic acid, C18:3 trans, arachidic acid, palmitic acid, linoleic acid, behenic acid, lignoceric acid, nervonic acid), glucosinolates (4-hydroxyglucobrassicin, glucobrassicin), other compounds (sinapine, ferulic acid, coumaric acid, phytic acid). **Treated**: proximates (crude fat, protein, crude fibre), amino acids (glycine, alanine, tyrosine, tryptophan, aspartic acid), vitamins (α-tocopherol, phylloquinone), minerals (phosphorus, magnesium, iron, potassium, zinc), sterols (brassicasterol, cholesterol, campesterol, stigmasterol, Δ-5 avenaterol, total phytosterols, 24-methylene cholesterol, Δ-7 stigmastenol), fatty acids (α-linolenic acid, C18:3 trans, arachidic acid, palmitic acid, linoleic acid, behenic acid, lignoceric acid, nervonic acid), glucosinolates (epi-progoitrin, glucobrassicin), other compounds (sinapine, ferulic acid, coumaric acid, phytic acid).

[e]Endpoints with significant differences between the oilseed rape LBFLFK and its conventional counterpart and falling in equivalence category IV. Both treated and not treated: calcium, palmitoleic acid, stearic acid, oleic acid, gondoic acid, ω6-eicosadienoic acid and total trans-fatty acids. Estimated means are reported for these endpoints in Table 5.

The GMO Panel assessed all significant differences between oilseed rape LBFLFK and its conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed in the set of non-GM reference varieties. Quantitative results for the endpoints showing significant differences between oilseed rape LBFLFK and its conventional counterpart and falling under category IV are given in Table 5. Owing to the introduced genetic modification, several new fatty acids were identified in oilseed rape LBFLFK that were absent in the conventional counterpart and in the non-GM reference varieties. These fatty acids are also listed in Table 5.

[16]Proximates and fibre content (ash, carbohydrates, moisture, protein, crude fat, crude fibre, acid detergent fibre and neutral detergent fibre), minerals (calcium, phosphorus, potassium, magnesium, iron, manganese, zinc), vitamins (total tocopherol, α-tocopherol, β-tocopherol, δ-tocopherol, γ-tocopherol, 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, palmitoleic acid, stearic acid, cis-vaccenic acid, oleic acid, linoleic acid, α-linolenic acid, arachidic acid, gondoic acid, ω6-eicosadienoic acid, behenic acid, lignoceric acid, nervonic acid, total trans fatty acids), C18:3 trans, sterols (24-methylene cholesterol, cholesterol, β-sitosterol, Δ-7 stigmastenol, brassicasterol, Δ-5 avenasterol, stigmasterol, campesterol, total phytosterols), glucosinolates (progoitrin, 4-hydroxyglucobrassicin, glucoalyssin, gluconasturtiin, glucobrassicin, glucobrassicanapin, epi-progoitrin, gluconapin, total glucosinolates), other compounds (phytic acid, ferulic acid, coumaric acid, sinapine).

TABLE 5 Quantitative results (estimated means and equivalence limits) for compositional endpoints in seeds that are further assessed based on the results of the statistical analysis. Fatty acids only present in oilseed rape LBFLFK but neither in the conventional counterpart nor in the non-GM reference varieties are also shown.

	Oilseed rape LBFLFK[a]			Non-GM reference varieties	
Endpoint[b]	Not treated	Treated	Conventional counterpart	Mean	Equivalence limits
Calcium (%)	0.271*	0.272*	0.300	0.395	0.319–0.470
C16:1ω7 (palmitoleic acid)	0.189*	0.188*	0.275	0.222	0.204–0.241
C18:0 (stearic acid)	3.046*	3.051*	2.375	2.248	2.086–2.409
C18:1ω9 (oleic acid, OLA)	29.256*	29.051*	58.799	66.450	45.484–87.416
C20:1ω9 (gondoic acid)	0.751*	0.751*	1.061	1.176	0.873–1.478
C20:2ω6 (ω6-eicosadienoic acid)	0.110*	0.109*	0.056	0.055	0.019–0.091
Total trans-fatty acids	0.164*	0.153*	0.105	0.084	0.047–0.121
Fatty acids only present in oilseed rape LBFLFK					
C18:2ω9 (octadecadienoic acid)	1.199	1.209	< LOQ	< LOQ	n.a.
C18:3ω6 (γ-Linolenic acid, GLA)	2.140	2.164	< LOQ	< LOQ	n.a.
C18:4ω3 (stearidonic acid, SDA)	0.332	0.340	< LOQ	< LOQ	n.a.
C20:2ω9 (eicosadienoic acid)	0.293	0.297	< LOQ	< LOQ	n.a.
C20:3ω3 (eicosatrienoic acid)	0.052	0.051	< LOQ	< LOQ	n.a.
C20:3ω6 (dihomo-γ-linolenic acid, DGLA)	4.708	4.739	< LOQ	< LOQ	n.a.
C20:4ω3 (bishomostearidonic acid)	1.958	1.982	< LOQ	< LOQ	n.a.
C20:4ω6 (arachidonic acid, ARA)	1.994	2.000	< LOQ	< LOQ	n.a.
C20:5ω3 (eicosapentaenoic acid, EPA)	4.597	4.673	< LOQ	< LOQ	n.a.
C22:4ω3 (docosatetraenoic acid)	0.980	0.974	< LOQ	< LOQ	n.a.
C22:4ω6 (adrenic acid)	0.525	0.530	< LOQ	< LOQ	n.a.
C22:5ω3 (clupanodonic acid, DPA)	2.537	2.583	< LOQ	< LOQ	n.a.
C22:6ω3 (docosahexaenoic acid, DHA)	0.385	0.391	< LOQ	< LOQ	n.a.

Abbreviations: LOQ, Limit of quantification (0.05% total fatty acids); n.a., not applicable.

[a]For the oilseed rape LBFLFK, significantly different values are marked with an asterisk. The outcome of the test of equivalence was category IV for both treated and not treated LBFLFK (dark grey). Treated: treated with the intended herbicide; not treated: treated only with conventional herbicides (see Section 3.4.4.3).

[b]Fatty acids are expressed as % total fatty acid content.

3.4.7 | Conclusion on 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 are appropriate to support the comparative analysis.

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

- None of the differences identified in agronomic and phenotypic characteristics between oilseed rape LBFLFK and the non-GM comparator needs further assessment regarding potential environmental impact except for germination of harvested seeds which is assessed in Section 3.6.1.1.
- None of the differences identified in seed composition between oilseed rape LBFLFK and its conventional counterpart needs further assessment regarding food and feed safety, except for calcium, palmitoleic acid, stearic acid, OLA, gondoic acid, ω6-eicosadienoic acid and TFA, which are assessed in Section 3.5. The food and feed safety assessment in Section 3.5 also included the fatty acids only present in oilseed rape LBFLFK as a result of its genetic modification (see Table 5).

3.5 | Food/feed safety assessment[17,18]

EFSA received a full-scope application for oilseed rape LBFLFK, which has an altered fatty acid profile (see Section 1.1).

[17]Dossier: Part II, sections 1.1, 1.4, 1.5, 1.6, 2, 3, 4 and 7; additional information: 2/7/2020, 30/9/2020, 8/12/2020, 24/2/2021, 28/4/2021, 23/8/2021, 12/1/2022, 3/5/2022, 6/4/2023, 30/11/2023, 3/5/2024 and 3/10/2024; spontaneous information: 11/10/2022, 31/7/2023, 11/10/2023, 15/12/2023 and 12/06/2025.

[18]In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed with diets containing defatted meal and refined, bleached, and deodorised (RBD) oil derived from oilseed rape LBFLFK (see Annex for details).

A full-scope application requires a risk assessment to cover all potential uses of oilseed rape LBFLFK, i.e. import and processing, and food and feed uses, mirroring the range of uses applicable to conventional oilseed rape. However, in this particular case the assessment could not be conducted as a standard full-scope application for several reasons:

a. The majority of newly expressed proteins in oilseed rape LBFLFK are membrane bound and, because of this, technically difficult to isolate, characterise and test according to current requirements (Regulation (EU) No 503/2013).[3] Furthermore, a history of safety use for consumption (HoSU) could not be documented for these proteins;
b. The substantially different composition in the fatty acid profile of oilseed rape LBFLFK as compared to the conventional counterpart requires a modification of the standard approach usually followed to nutritionally assess GM crops. The uses of oilseed rape LBFLFK, due to its particular composition in fatty acids, will differ entirely from those of its conventional counterpart. This composition determines new uses (intended uses) that were provided by the applicant and then considered during the assessment, e.g. its use as a potential source of EPA and DHA;
c. Furthermore, available data indicate that the fatty acid profile of RBD LBFLFK oil may substantially vary depending on several biological factors, including the genetic background of the oilseed rape plant according to the additional information provided by the applicant.[19] This reported high variability limits the extent to which the current compositional data set can be considered representative for a full-scope assessment, and adds further complexity to the assessment, as described in Section 3.5.2.1.

Based on the particular characteristics of oilseed rape LBFLFK, the applicant proposed in the original dossier intended uses for different products, i.e. defatted meal for animals, and oil for humans and aquaculture. The following sections outline the key scientific data and considerations underlying the protein and fatty acid safety assessment of oilseed rape LBFLFK for both human and animal health, together with the conclusions of the risk assessment. In the case of the human nutritional assessment, the applicant provided detailed information on the intended uses of the RBD oil, and submitted a list of food products with a predefined composition of relevant fatty acids (see Appendix C). This dedicated approach allowed the GMO Panel to conclude that the consumption of RBD oil from oilseed rape LBFLFK is safe and does not represent any nutritional concern in humans (Section 3.5.2.1).

To preserve transparency while focusing the scientific opinion on the most relevant data and conclusions, the standard, full-scope food/feed assessment and the complete set of data and risk assessment considerations required under Regulation (EU) No 503/2013[3] are provided in Annex A.

3.5.1 | Protein safety assessment

In accordance with current requirements described in Regulation (EU) No 503/2013,[3] toxicological and allergenicity assessments of NEPs, referred hereafter as 'protein safety', follow a case-by-case approach. When there is well-documented evidence of a HoSU for both the plant and the NEPs in food and/or feed, specific testing may not be necessary.

The GMO Panel evaluated the information provided by the applicant in support of the claimed HoSU. The GMO Panel concluded that the HoSU as food and feed of the desaturases and elongases newly expressed in oilseed rape LBFLFK was not adequately documented. The source organisms of these enzymes are not commonly used in food or feed, and dietary exposure to these or similar desaturases and elongases is expected to be minimal from such sources.

Although fatty acid desaturases and elongases are widely present in conventional foods and feeds, these enzymes newly expressed in oilseed rape LBFLFK exhibit low primary amino acid sequence identity with those found in traditional food and feed sources. In addition, the 3D structural analysis provided by the applicant was insufficient to conclude on safety, mainly because the selected comparator proteins used were not documented in food and feed, and due to unresolved uncertainties regarding the relevance of the in silico tools used for the risk assessment. Even though homologous desaturases and elongases are expressed in animals that are part of the human diet (e.g. Atlantic salmon), the highest levels of gene expression were only determined by transcript levels and were mainly observed in tissues such as the heart, brain and intestine, which are organs not typically consumed. Experimental measurement of comparator protein concentrations in edible tissues and derived food/feed products have not been provided. As a result, dietary exposure to these enzymes cannot be reliably estimated, and therefore cannot support a conclusion regarding the HoSU of the desaturases and elongases newly expressed in oilseed rape LBFLFK.

In accordance with Regulation (EU) No 503/2013,[3] the absence of a duly documented HoSU of NEPs necessitates specific in vitro and in vivo studies, including a repeated-dose 28-day oral toxicity study. However, such studies were not provided for the individual desaturases and elongases newly expressed in oilseed rape LBFLFK. These proteins are integral membrane-bound enzymes, making them difficult to isolate, concentrate, and quantify from plant tissues. Additionally, their intractable nature poses significant challenges for expression in heterologous systems for their use in standard protein safety assessments.

[19]Additional information: 3/10/2024.

The applicant submitted an alternative 28-day oral toxicity study using PPPs, but it was not considered fit for hazard identification. The administered doses of the individual desaturases and elongases were too low to be informative. For example, the highest dose was 6.5 mg/kg body weight for D6E(Tp), which is substantially below the standard 1000 mg/kg body weight dose typically used in 28-day toxicity studies. Regarding in vitro studies, the GMO Panel identified limitations in the protein degradation studies submitted by the applicant. The full interpretation of the degradation readouts for some of the NEPs and their fragments was not possible. Moreover, not all NEPs were studied in vitro. Currently, there are no validated or regulatory-accepted methods available to assess the safety of complex protein mixtures such as the NEPs from oilseed rape LBFLFK present in the PPP preparations.

The GMO Panel recognises the challenges involved in conducting protein safety studies for certain proteins, such as membrane-bound proteins expressed in oilseed rape LBFLFK (EFSA GMO Panel, 2022a, 2022b, 2025). Significant uncertainties remain in the assessment of these NEPs, particularly due to the lack of a documented HoSU, the absence of experimental data aligned with current regulatory requirements, and the applicant's reliance on in silico tools, which currently lack regulatory acceptance. To address these challenges and support progress in the prediction of toxicity and allergenicity, the GMO Panel recently published a scientific opinion outlining lessons learned and future needs in protein safety assessment (EFSA GMO Panel, 2025). For example, potential future strategies for assessing complex cases, such as NEPs in oilseed rape LBFLFK, could include: (i) analyses based on phylogenetic relationships and evolutionarily conserved protein clusters; and/or (ii) safety evaluations on specific intended uses through targeted, product-driven investigations (EFSA GMO Panel, 2025).

Based on the information provided by the applicant, the GMO Panel concludes that the safety of the elongases and desaturases newly expressed in oilseed rape LBFLFK cannot be established. For further details on the toxicological and allergenicity assessments, see Annex A.

The GMO Panel was able to establish the safety of AHAS(At) based on the following considerations: (i) the endogenous AHAS protein counterpart in the recipient *B. napus* shares 88% identity with AHAS(At); (ii) AHAS proteins expressed in other commonly consumed crops show up high sequence identity; and (iii) a highly similar AHAS(At) protein (99% identical) has previously been evaluated by the GMO Panel, with no safety concerns identified (see Annex A).

3.5.2 | Fatty acid nutritional assessment

Table 5 in Section 3.4.6 shows the different fatty acids considered during the nutritional assessment. The biological relevance of these fatty acids, the role of oilseed rape as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment.

3.5.2.1 | *Human nutritional assessment of fatty acids*[20]

At present, oil is almost the only food commodity derived from oilseed rape regularly consumed by the European population, although other food commodities from oilseed rape (from non-GM sources) were approved as novel food in recent years, i.e. protein isolates (EFSA NDA Panel, 2013) and oilseed rape powder (EFSA NDA Panel, 2020). Because of this and the fact that the genetic modification in oilseed rape LBFLFK mainly results in the modification of its fatty acid profile, the human nutritional assessment focused on the consumption of refined, bleached and deodorised (RBD) oil produced from the GM crop (hereinafter RBD LBFLFK oil).

Comparison between fatty acid composition in seeds and RBD oil

As the first step, the composition of the seeds was compared to that of the RBD LBFLFK oil. As shown in Table 6, the modified fatty acid composition of oilseed rape LBFLFK seeds is almost entirely reflected in the composition of the RBD LBFLFK oil. The refining process to produce RBD oil led to a small increase in the percentage of total TFAs as compared to the seeds; this increase was observed in both the RBD LBFLFK oil and the oil produced from its conventional oilseed rape. Additionally, two PUFAs, C20:3ω9 (mead acid) and C22:5ω6 (osbond acid), were only identified in RBD LBFLFK oil, both at levels well below 1% total fatty acids. These two fatty acids were also considered during the nutritional assessment.

[20]Dossier: Part II, sections 1.1, 1.6, 2, 3, 4 and 7; additional information: 30/9/2020 and 28/4/2021; spontaneous information: 11/10/2022.

TABLE 6 Percentage of relevant fatty acids in LBFLFK oilseed rape, in the refined, bleached and deodorised (RBD) oil produced from oilseed rape LBFLFK (RBD LBFLFK oil) and in RBD oil produced from conventional oilseed rape.

		(% total fatty acids)		
		Oilseed rape LBFLFK[b]		
Common name	Systematic name[a]	Seeds[c]	RBD oil[d]	RBD oil from conventional oilseed rape[e]
C16:1ω7 (palmitoleic acid)	cis-9-Hexadecenoic acid	0.188	0.180	0.246
C18:0 (stearic acid)	Octadecanoic acid	3.051	3.107	2.299
C18:1ω9 (oleic acid, OLA)	cis-9-Octadecenoic acid	29.051	30.323	59.296
C20:1ω9 (gondoic acid)	cis-11-Eicosenoic acid	0.751	0.803	1.129
C20:2ω6 (ω6-eicosadienoic acid)	All-cis-11,14-Eicosadienoic acid	0.109	0.114	0.058
Total trans-fatty acids	Total trans-fatty acids	0.153	0.617	0.627
C18:2ω9 (isolinoleic acid)	All-cis-6,9-Octadecadienoic acid	1.209	1.407	0.067
C18:3ω6 (γ-Linolenic acid, GLA)	All-cis-6,9,12-Octadecatrienoic acid	2.164	2.153	–
C18:4ω3 (stearidonic acid, SDA)	All-cis-6,9,12,15-Octadecatetraenoic acid	0.340	0.307	–
C20:2ω9 (ω9-eicosadienoic acid)	All-cis-8,11-Eicosadienoic acid	0.297	0.359	0.033
C20:3ω3 (eicosatrienoic acid)	All-cis-11,14,17-Eicosatrienoic acid	0.051	0.061	–
C20:3ω6 (dihomo-γ-linolenic acid, DGLA)	All-cis-8,11,14-Eicosatrienoic acid	4.739	4.880	–
C20:4ω3 (bishomostearidonic acid)	All-cis-8,11,14,17-Eicosatetraenoic acid	1.982	1.885	–
C20:3ω9 (mead acid)	All-cis-5,8,11-Eicosatrienoic acid	–	0.047	–
C20:4ω6 (arachidonic acid, ARA)	All-cis-5,8,11,14-Eicosatetraenoic acid	2.000	1.724	–
C20:5ω3 (eicosapentaenoic acid, EPA)	All-cis-5,8,11,14,17-Eicosapentaenoic acid	4.673	4.081	–
C22:4ω3 (docosatetraenoic acid)	All-cis-7,13,16,19-Docosatetraenoic acid	0.974	1.006	–
C22:4ω6 (adrenic acid)	All-cis-7,10,13,16-Docosatetraenoic acid	0.530	0.518	–
C22:5ω3 (clupanodonic acid, DPA)	All-cis-7,10,13,16,19-Docosapentaenoic acid	2.583	2.191	–
C22:5ω6 (osbond acid)	All-cis-4,7,10,13,16-Docosapentaenoic acid	–	0.056	–
C22:6ω3 (docosahexaenoic acid, DHA)	All-cis-4,7,10,13,16,19-Docosahexaenoic acid	0.391	0.352	–

Abbreviation: RBD oil, refined, bleached and deodorised oil.

[a]Adapted from IUPAC.

[b]Oilseed rape LBFLFK not treated with the intended herbicide.

[c]Fatty acid percentage as determined for the comparative compositional analysis (see Section 3.4.6).

[d]Fatty acid percentage in RBD oil from oilseed rape LBFLFK as described in study report 201,704–002 (mean value of 3 technical replicates of a single pooled sample).

[e]Fatty acid percentage in RBD oil from the conventional oilseed rape variety Kumily as described in study report 201704–002 (mean value of 3 technical replicates of a single pooled sample).

Together with the presence of EPA and DHA, the RBD LBFLFK oil also contains in relatively high amounts other PUFAs such as C20:3ω6 (dihomo-γ-linolenic acid, DGLA), C22:5ω3 (clupanodonic acid, DPA), C18:3ω6 (γ-linolenic acid, GLA) and C20:4ω6 (arachidonic acid, ARA). The GMO Panel noted that the levels of EPA and DHA in the RBD LBFLFK oil are much lower than those in menhaden oil (~4% vs. 17.5% total fatty acids for EPA and ~0.4% vs. 13% total fatty acids for DHA) and other fish oils. Overall, the RBD LBFLFK oil contains twice the amount of ω3 and ω6 fatty acids as compared to oil from its conventional counterpart (Andre et al., 2019). Furthermore, there is a decrease of ~50% in OLA content, the most abundant fatty acid in conventional oilseed rape.

The safety of a total of 20 fatty acids was assessed: 15 fatty acids only present in the RBD LBFLFK oil and 5 fatty acids with altered levels as compared to oil from conventional oilseed rape. Additionally, the percentage of total TFAs in the RBD LBFLFK oil was also assessed.

Three fatty acids (palmitoleic acid, OLA and gondoic acid) were at significant lower levels in the RBD LBFLFK oil as compared to its conventional counterpart oil. As regards OLA, levels decreased around 50%. However, OLA is not an essential fatty acid since it is synthesised by the body. OLA is also present in many other foods, particularly vegetable oils, at relatively high levels (EFSA NDA Panel, 2010). Gondoic acid is a very minor fatty acid in oilseed rape oil (< 1% total fatty acids), found at low levels in milk and dairy products (Devle et al., 2012). It is not identified as an essential fatty acid. Palmitoleic acid is usually found at trace levels in commonly consumed vegetable oils. Therefore, the decrease in these three fatty acids does not represent a nutritional concern.

Similar percentages of total TFAs were found in the RBD LBFLFK oil and the conventional oil; in both cases the levels increased during the refining process. Adverse health effects related to the presence of total TFAs in the RBD LBFLFK oil are

not expected due to their low amounts (~0.6% total fatty acids). Commission Regulation 2019/649[21] specifies that a maximum of 2 grams/100 grams fat of TFAs other than TFAs naturally occurring in fat of animal origin is allowed in food intended for the final consumer and food intended for supply to retail.

The nutritional assessment followed two different approaches for the fatty acids with either increased levels or only present in the RBD LBFLFK oil. For 12 fatty acids where no reference doses were available, the assessment was based on a weight of evidence approach, considering the absence/presence of toxicological hazards and a HoSU. For five fatty acids, the nutritional assessment was conducted comparing their intake estimations to doses without adverse effects available in the scientific literature: GLA, C18:4ω3 (stearidonic acid, SDA), EPA, DHA and DPA.

Nutritional assessment based on a weight of evidence approach

a. Seven fatty acids were present at very small amounts in the RBD LBFLFK oil (< 1% total fatty acids): C20:2ω6 (ω6-eicosadienoic acid), C20:2ω9 (ω9-eicosadienoic acid), C20:3ω3 (eicosatrienoic acid), mead acid, C22:4ω3 (docosatetraenoic acid), C22:4ω6 (adrenic acid) and osbond acid. To support the HoSU of these eight fatty acids, the applicant provided evidence from scientific literature on their presence in habitually consumed foods. Furthermore, the applicant also analysed different foods, including vegetable oils (see study report 201703-004R). As an example, docosatetraenoic acid has been reported in wild sea bass (Alasalvar et al., 2002) and it is also found in different fish oils, including menhaden oil. Adrenic acid was quantified in shrimps and both marine and freshwater fish (e.g. mackerel, eel, carps) (Li et al., 2011), as well as in meat products and fish oils. Taking into account this information, the GMO Panel considered that the presence of these eight fatty acids in the RBD LBFLFK oil does not represent a nutritional concern.
b. Five fatty acids were present in the RBD LBFLFK oil at concentrations > 1% total fatty acids: stearic acid, C18:2ω9 (isolinoleic acid), DGLA, C20:4ω3 (bishomostearidonic acid) and ARA. Their concentrations range between ~1.4% total fatty acids for isolinoleic acid and ~5% total fatty acids for DGLA. As for the other eight fatty acids, no toxicological hazards are described in the literature. In all cases, the HoSU for these fatty acids was supported with both published scientific data and analytical data produced by the applicant.

DGLA has been reported to be present in animal tissues as a minor component (FAO, 2010); the applicant also quantified this fatty acid in several animal derived foods such as liver (up to 6.8% total fatty acids), dairy products (cheese, butter), fish oils and *Mortierella alpina* oil (~3.5% total fatty acids), a fungal oil authorised as novel food ingredient in 2008.[22] Stearic acid is a common fatty acid present in both vegetable and animal fats. For this fatty acid, there was a small increase as compared to the conventional counterpart (from ~2.3% to ~3.1% total fatty acids). These levels are similar to those described in other vegetable oils; overall, the amount of saturated fatty acids in the RBD LBFLFK oil was only 1% higher than in the oil produced from the conventional counterpart. Bishomostearidonic acid was detected at levels of ~2% total fatty acids. This ω3 fatty acid has been reported in animal tissues and fish oils (Ghioni et al., 2002) as well as in brown seaweeds (Foseid et al., 2017). It is also present in menhaden oil at levels around 1.5% total fatty acids. Additionally, it was detected in butter and in *Mortierella alpina* oil among the food samples analysed by the applicant. ARA is an ω6 fatty acid detected in the RBD LBFLFK oil at levels around 1.7% total fatty acids. This fatty acid is a typical component of animal fat. Among the food samples analysed by the applicant, it was detected across different food categories; in meat samples the levels varied between 0.04% and 9.8% total fatty acids. The fungal *Mortierella alpina* oil (~3.5% total fatty acids), authorised as novel food ingredient in 2008 as mentioned above, is an ARA-rich oil (≥ 40%). Isolinoleic acid, an ω9 fatty acid, is present at different levels in a broad variety of commonly consumed fish (e.g. gilthead, sea bass, etc.), crustaceans (e.g. blue crab) and molluscs (e.g. mussels), as well as fish oils (Beccaria et al., 2015; Çelik et al., 2004; Costa et al., 2017; Grigorakis et al., 2010). The analyses carried out by the applicant on different foods also identified isolinoleic acid in meat samples, cheese and butter. Based on this evidence, the GMO Panel concluded that no nutritional concern is identified linked to the presence of any of these five fatty acids in the RBD LBFLFK oil.

Nutritional assessment based on intake estimations

The nutritional assessment of GLA, SDA, EPA, DHA and DPA in the RBD LBFLFK oil followed a stepwise process (tiered approach). The initial intake scenario consisting of a full replacement of all vegetable oils consumed in Europe (not only oil from oilseed rape) by the RBD LBFLFK oil was considered unrealistic based on its specific composition in fatty acids that make it technologically unsuitable for many uses. Additionally, oilseed rape LBFLFK is expected to be cultivated as a specialty canola variety, so the production is not expected to cover current consumption of vegetable oil in Europe. Therefore, the GMO Panel did not consider this intake scenario as adequate for the assessment.

[21]Commission Regulation (EU) 2019/649 of 24 April 2019 amending Annex III to Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards trans-fat, other than trans-fat naturally occurring in fat of animal origin.

[22]Commission Decision of 12 December 2008 authorising the placing on the market of arachidonic acid-rich oil from *Mortierella alpina* as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council.

In a higher tier, the assumption that oil from oilseed rape might be replaced by the RBD LBFLFK oil was assessed. Applicant provided an intake replacement scenario using different data sources to characterise the consumption of oil from oilseed rape in the US population (e.g. FAOSTAT Food Balance Sheets). This information was deemed not adequate for the nutritional assessment of the RBD LBFLFK oil because (1) the consumption data are not representative of the consumption in Europe, and (2) the specific composition of the RBD LBFLFK oil implies different uses as compared to those of oil from oilseed rape precluding the replacement. Therefore, the GMO Panel did not consider this intake scenario as adequate for the assessment.

Following a GMO Panel request based on the composition of the RBD LBFLFK oil, the applicant proposed food uses based on those authorised by the US Food and Drug Administration (FDA) for menhaden oil (see Appendix C).[23] The proposed amounts of RBD LBFLFK oil aim at having specific maximum concentrations of fatty acids in the different foods, in particular of EPA and DHA. This scenario is based on the Codex Alimentarius statement proposing that when the genetic modifications result in food products with a composition that significantly differs from their conventional counterparts (e.g. RBD LBFLFK oil), dietary intake estimations should consider the nutrient composition of the GM food products when selecting the foods likely to be displaced (Codex Alimentarius, 2009). Neither food supplements nor foods specifically manufactured for infants and young children are included among the intended uses provided by the applicant.[24] The applicant intends to add different amounts of RBD LBFLFK oil to selected foods to reach a specific concentration of fatty acids, in particular EPA and DHA. To account for the possible variability of fatty acids in the RBD LBFLFK oil based on different factors as described by the applicant (e.g. genetic background of the oilseed rape plant),[19] the final maximum concentrations of GLA, SDA, EPA, DHA and DPA in the proposed foods are those used as reference for the nutritional assessment (Appendix C). Should the applicant request additional food uses in the future (i.e. beyond its use as ingredient in the selected food categories), the current nutritional assessment will have to be revisited to conclude on the safety of the RBD LBFLFK oil.

The intakes were estimated by combining consumption data[25] reported for conventional foods where RBD LBFLFK oil is intended to be added and the maximum concentrations of the fatty acids in each food as proposed by the applicant. The food categories where menhaden oil is authorised were mapped into the different food categories codified under the FoodEx2 system in the EFSA consumption database (Appendix C). Summary statistics of consumption were used for calculating dietary exposure to GM food components (EFSA, 2019b). A scenario with 100% replacement of conventional foods by RBD LBFLFK oil-containing foods was considered.

The GMO Panel notes that, at present, no tolerable upper intake levels (UL) are set for ω3/ω6 PUFAs since no consistent evidence exists that the intake of any of these fatty acids has detrimental effects on health for any population group (EFSA NDA Panel, 2010). The EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) considers that supplemental intakes of EPA and DHA combined at doses up to 5 g/day (without specifying EPA:DHA ratios), and supplemental intakes of EPA alone up to 1.8 g/day do not raise safety concerns for the adult population. Supplemental intakes of DHA alone up to about 1 g/day are also considered as not raising safety concerns for the general population (EFSA NDA Panel, 2012). For the nutritional assessment of SDA and GLA, reference doses of 4.2 g/day (Lemke et al., 2010) and 2.8 g/day (Kenny et al., 2000; Zurier et al., 1996) were selected, respectively. These are maximum doses used in human studies without reports of adverse effects and were already used in the past by the GMO Panel as reference points (EFSA GMO Panel, 2014, 2021). The intake estimations were then compared to these doses in order to conclude on potential nutritional concerns linked to the presence of these five fatty acids in the RBD LBFLFK oil.

The different doses were converted into mg/kg body weight (bw) day using a default value of 70 kg (EFSA Scientific Committee, 2012) to allow comparison with the fatty acid intake across different age classes. For SDA and GLA, the maximum intake estimates for high consumers were 4 mg/kg bw day and 30 mg/kg bw day, respectively (see Table 7 below). These estimates are below the reference doses without adverse effects for these compounds, 40 mg/kg bw day for GLA and 60 mg/kg bw day for SDA. Therefore, no nutritional concerns were identified to the presence of these two fatty acids in the RBD LBFLFK oil.

The intake estimations for EPA and DHA included DPA, another long-chain ω3 PUFA which is an intermediary product between EPA and DHA. As for these two PUFA, no UL has been set by any authoritative body for DPA (EFSA NDA Panel, 2012). The intake estimations for EPA + DHA + DPA were overall well below the reference dose (71.4 mg/kg bw day), although high intakes were above in few dietary surveys (Table 7). The uncertainties linked to the methodology used in the intake assessment and the assumptions made were taken into account when interpreting this outcome and concluding on the nutritional assessment. These uncertainties mainly refer to (1) the conservatism of using summary statistics of consumption instead of individual data, and (2) the overly conservative assumption on the presence of RBD LBFLFK oil in all intended uses and the 100% replacement of all conventional foods. The uncertainties linked to the methodology as well as the assumptions overestimate the intakes. Based on this and considering the scientific opinion issued by the NDA Panel (EFSA NDA Panel, 2010), the GMO Panel concludes that the presence of EPA, DHA and DPA in RBD LBFLFK oil does not represent a nutritional concern for humans under the proposed conditions of use.

[23] https://www.govinfo.gov/app/details/CFR-2022-title21-vol3/CFR-2022-title21-vol3-sec184-1472. Accessed on 21 August 2023.

[24] https://food.ec.europa.eu/safety/labelling-and-nutrition/specific-groups/food-infants-and-young-children_en.

[25] Summary statistics of consumption from the EFSA Comprehensive European Food Consumption Database (EFSA Consumption database). The EFSA Consumption Database is a source of information on food consumption across the European Union (EU). http://www.efsa.europa.eu/en/food-consumption/comprehensive-database.

TABLE 7 Range of dietary intakes for selected fatty acids across population groups based on the intended uses (selected foods as proposed by the applicant) of RBD oil produced from oilseed rape LBFLFK (see Appendix C).

		Dietary intake (mg/kg bw per day)					
		EPA + DHA + DPA[b]		SDA[c]		GLA[d]	
Age classes[a]	N	Mean	High intake	Mean	High intake	Mean	High intake
Infants	13	4–34	6–88	< 1–2	< 1–4	1–11	2–28
Toddlers	20	24–71	37–93	1–3	2–4	8–23	12–30
Other children	30	31–58	44–84	1–3	2–4	10–19	14–27
Adolescents	30	16–33	22–43	1–2	1–2	5–11	7–14
Adults	35	11–22	16–36	1	1–2	4–7	5–12
Elderly	25	9–17	12–27	< 1–1	1	3–6	4–9
Very elderly	17	9–19	13–27	< 1–1	1	3–6	4–9
Pregnant and lactating women	7	12–17	15–23	1	1	4–6	5–7

[a]Infants: < 12 months old; Toddlers: ≥ 12 months to < 36 months old; Other children: ≥ 36 months to < 10 years old; Adolescents: ≥ 10 years to < 18 years old; Adults: ≥ 18 years to < 65 years old; Elderly: ≥ 65 years to < 75 years old; Very elderly: ≥ 75 years old.

[b]EPA: eicosapentanoic acid; DHA: docosahexanoic acid; DPA: docosapentaenoic acid.

[c]SDA: stearidonic acid.

[d]GLA: γ-Linolenic acid.

Potential hazards have been identified in the literature linked to the use of oils rich in PUFAs for high-temperature frying practices (~180°C) due to the generation of lipid oxidation products such as aldehydes and epoxy-fatty acids (Vieira et al., 2017; Grootveld et al., 2022). The use of the RBD LBFLFK oil as frying oil is not one of the intended uses identified by the applicant and, therefore, this use was not considered during the nutritional intake assessment. However, one of the intended food uses of the RBD LBFLFK oil is as ingredient of fats and oils. Due to the added value and specific characteristics of the RBD LBFLFK oil, these fats and oils (e.g. margarines, butters, oils; see Appendix C for details) are not expected to have some of the uses that sometimes the conventional counterparts have (e.g. for frying). Considering the potential safety concerns identified for frying or other high-temperature uses of oils rich in PUFAs, these foods should not be used for high-temperature applications.

The potential additional contribution to the fatty acid intake via the use as food additive of the lecithin fraction from oilseed rape LBFLFK (by-product of oil refinement) was also assessed. The lecithin fraction of both the conventional oilseed rape and oilseed rape LBFLFK contains higher proportion of saturated and polyunsaturated fatty acids as compared to the crude oil, but a lower proportion of monounsaturated fatty acids. The applicant claimed that the use of lecithin from oilseed rape is much smaller compared to soybean lecithin. Taking into account that the use of lecithin is not considered as an intended use in the context of this application, the GMO Panel concludes that the potential contribution of the lecithin fraction from oilseed rape LBFLFK to the fatty acid intake would be negligible in the context of the intake scenario already considered.

In terms of fatty acid bioavailability, the applicant reviewed the available literature for EPA and DHA to assess whether the structure and stereochemical configuration of triacylglycerols containing these two PUFAs might differ between oilseed rape LBFLFK and conventional sources, and if this could have an impact on bioavailability. Human studies provided by the applicant show that the lipid backbone and stereochemical arrangement of the fatty acids have little or no impact on the bioavailability of EPA and DHA. Based on the current knowledge, the GMO Panel considers that potential differences in the structure and the stereochemical configuration of lipids containing EPA and DHA as compared to that in conventional sources do not represent a safety concern.

3.5.2.2 | *Animal nutritional assessment of fatty acids*

This section presents the outcome of the safety and nutritional assessment of fatty acids of oilseed rape LBFLFK, based on the consumption of relevant feed materials derived from this oilseed rape (e.g. LBFLFK oil and RBD LBFLFK oil,[26] full-fat rapeseed). Annex A provides further supplementary details of all the information considered during the assessment.

Terrestrial farmed and companion animals

For 15 fatty acids and total TFAs identified during the comparative assessment, either at levels significantly different from those in conventional oilseed rape grains/oil or present only in the GM crop/oil, no safety and nutritional concerns were identified for terrestrial farmed and companion animals. The fatty acids were palmitoleic acid, OLA, gondoic acid, stearic

[26]Both RBD and crude oils are used in animal nutrition. While RBD oils are generally preferred for their stability, consistency, and nutritional reliability, crude oils may also be used in feed formulations, for example when local availability, cost, or processing infrastructure favour their use, provided that the quality and safety are suitable for the target species and production system.

acid, ω6-eicosadienoic acid, GLA, stearidonic acid, eicosatrienoic acid, mead acid, bishomostearidonic acid, ARA, EPA, DPA, osbond acid and DHA. A comparative dietary intake assessment for these fifteen fatty acids and total TFAs shows that expected exposure levels fall within the ranges associated with commonly used fatty acid-rich feeds such as soybean oil or marine by-products (e.g. menhaden, salmon and anchovy oil) (see Annex A). Therefore, a HoSU is demonstrated.

However, for five fatty acids, the comparative dietary intake assessment indicates that their exposure levels exceed those typically associated with conventional fatty acid-rich feeds (see Annex A). These fatty acids are isolinoleic acid, ω9-eicosadienoic acid, DGLA, docosatetraenoic acid and adrenic acid. Following several requests asking to clarify and further support the HoSU of these five fatty acids in commercial/conventional diets,[27] the applicant submitted limited supporting literature to justify the HoSU in the diets of terrestrial farmed and companion animals, and did not provide any new data that could potentially change the overall safety and nutritional assessment.[28] Furthermore, no dedicated studies on the oils alone were provided to support the safety of these five fatty acids.

Based on the current evidence, the GMO Panel concludes that the safety of RBD LBFLFK oil for its use in terrestrial farmed and companion animals cannot be established.

Aquatic species

As observed for terrestrial farmed and companion animals, the HoSU for isolinoleic acid, ω9-eicosadienoic acid, DGLA, docosatetraenoic acid and adrenic acid was not demonstrated when conducting the safety and nutritional assessment for the use of LBFLFK oil in aquaculture.[27]

Unlike the assessment for terrestrial farmed and companion animals, in the case of the aquaculture assessment different feeding studies were available. Feeding studies with the LBFLFK oil were conducted in certain aquaculture species, enabling a partial assessment of safety and nutritional equivalence in the target animals (see Annex A). Some of these studies provided observations suggestive of potential adverse effects, including changes in health-related markers, pointing to pathways that may be worth exploring further to understand their causes, possibly linked to oxidative stress and inflammatory responses (Hong et al., 2022). Furthermore, data from feeding studies in crustaceans also suggested that high levels of LBFLFK oil replacement may result in reduced growth and potential nutritional deficiencies, possibly due to decreased levels of essential fatty acids, such as DHA (Vo et al., 2021; Weldon et al., 2023).

Moreover, the intended use of LBFLFK oil in aquaculture was not clearly defined. Specifically, there was no information provided on inclusion rates in diets for different species, or whether the oil is intended to directly replace existing fish oil products. In addition, the relationship between inclusion rates and the findings, such as those noted in rainbow trout (Hong et al., 2022), or the reported nutritional deficiencies in Pacific white shrimp (Vo et al., 2021; Weldon et al., 2023) were not fully addressed. No tolerable inclusion levels were proposed.

Based on the current information available, the GMO Panel concludes that the safety and nutritional adequacy of LBFLFK oil for its use in aquaculture cannot be established.

General remarks

Although the human nutritional assessment of the RBD LBFLFK oil was completed satisfactorily (see Section 3.5.2.1), the GMO Panel cannot establish the safety and nutritional adequacy for the use of LBFLFK oil in animal feed, including terrestrial farmed animals, companion animals and aquaculture species. In the case of humans, a comprehensive nutritional assessment was conducted considering a HoSU that relies on well-documented consumption of different foods containing the fatty acids under assessment and for which data were provided. In addition, realistic displacement scenarios, based on maximum fatty acid concentrations in a list of foods, allowed an adequate nutritional intake assessment, comparing intakes with different doses without adverse effects. In contrast, (food-producing) animals consume highly formulated diets in which specific fatty acids are delivered at consistent, often elevated, inclusion rates. All this renders exposure modelling and reference dose comparisons, as applied in human risk assessment, inadequate for feed safety assessment. The standardised feeding regimens of animal production require either a duly documented HoSU based on market relevant data or properly controlled feeding studies. In the absence of this information, the safety and nutritional adequacy of the oil and full-fat seeds derived from oilseed rape LBFLFK for its use in aquaculture and in terrestrial farmed and companion animals cannot be established.

The identified uncertainties related to the assessment of animal feed uses could be addressed providing a well-documented HoSU. This can be achieved demonstrating nutritional equivalence of the relevant GM feed materials with conventional feed materials in representative market contexts, supported by market-derived feed consumption data and typical inclusion rates. Robust nutritional assessments based on realistic feeding scenarios and representative feed formulations should reflect commercial practice and proposed inclusion/partial replacement levels.

[27]Additional information: 3/5/2022, 6/4/2023, 30/11/2023, 3/5/2024, 3/10/24.

[28]The applicant submitted spontaneous additional information to support the HoSU of these five fatty acids (received after the final ADR 17 (3/10/2024) that concluded the RA process after 5 years assessment). Upon review of this information, the GMO Panel found the provided methodology unclear and not fit-for-purpose as the selection criteria of the feed materials were neither justified nor representative of feed market relevance, the exposure scenarios were unrealistically high, and the selective coverage of only five fatty acids introduced a risk of internal bias.

If a HoSU cannot be documented, relevant and fit-for-purpose feeding studies in representative target species could be provided. Such studies could use inclusion rates aligned with commercial practices (including partial replacement scenarios), include appropriate control/reference diets, report core performance and health endpoints (and markers relevant to suspected mechanisms such as oxidative stress or inflammatory responses), have adequate replication and duration, and consider essential fatty acid status where relevant.

3.5.3 | Calcium nutritional assessment

The human and animal nutritional assessment related to the observed decrease of calcium levels in oilseed rape LBFLFK as compared to its conventional counterpart is described in Annex A. Based on the available information, the GMO Panel concludes that the observed decrease in the levels of calcium in oilseed rape LBFLFK seeds does not raise any nutritional concern in humans and animals.

3.5.4 | Post-market monitoring of GM food/feed

The GMO Panel notes that the pre-market risk assessment conducted for oilseed rape LBFLFK, in particular for RBD LBFLFK oil, assumed a 100% replacement of conventional foods by the foods proposed by the applicant containing the RBD LBFLFK oil (intended uses). Therefore, the outcome of the safety assessment will be hardly impacted by real data on the consumption of foods containing the RBD LBFLFK oil, provided that the conditions of use (authorised uses) are those considered during the pre-market risk assessment.

In accordance with Article 6(5)(e) of Regulation (EC) No 1829/2003 and as indicated in Commission Implementing Regulation (EU) No 503/2013,[3] notwithstanding the fact that the safety of the genetically modified food is demonstrated, post-market monitoring (PMM) is appropriate to confirm the expected consumption and the application of conditions of uses. EFSA recommends that the PMM proposal should initially focus on the collection of import data to Europe of oil produced from oilseed rape LBFLFK (RBD LBFLFK oil) and of any of the food commodities considered during the assessment where the RBD LBFLFK oil is intended to be added as ingredient (see Section 3.5.2.1 and Appendix C). If imports are identified, the PMM should focus on confirming that (1) only the foods containing RBD LBFLFK oil considered in the pre-market risk assessment enter the European market, and (2) the maximum fatty acids concentrations in these foods are those considered in the pre-market risk assessment. Because the GMO Panel cannot establish the safety and nutritional adequacy of oilseed rape LBFLFK for terrestrial animals and aquatic species, it is also not in the position to comment on PMM of the GM feed.

3.5.5 | Labelling

In accordance with Articles 13(2)(a) and 25(2)(c) of Regulation (EC) No 1829/2003, the applicant proposed that oilseed rape LBFLFK should have specific labelling: '*Genetically modified rapeseed with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)*'. The GMO Panel agrees that specific labelling for oilseed rape LBFLFK is needed based on its particular composition in fatty acids and the intended uses that will differ from those of its conventional counterpart (see Section 3.4.6). However, the GMO Panel considers that the proposed labelling is not scientifically correct as it does not fully characterise the compositional changes in the GM crop. Together with the presence of EPA (~4.7% total fatty acids) and DHA (~0.4% total fatty acids), other PUFAs such as DGLA (~4.7% total fatty acids), DPA (~2.6% total fatty acids), GLA (~2.2% total fatty acids) and ARA (~2% total fatty acids) are also present in relatively high amounts in oilseed rape LBFLFK. Furthermore, as compared to its conventional counterpart, there is a decrease of ~50% in oleic acid content, the most abundant fatty acid in conventional oilseed rape. The labelling should be adjusted to be scientifically correct.

Additionally, considering the potential safety concerns identified for frying or other high-temperature uses of oils rich in PUFAs, appropriate labelling of certain food uses of RBD LBFLFK oil (e.g. margarines, butters, oils; see Appendix C for details) could help ensure that they are not used for these purposes (see Section 3.5.2.1).

3.5.6 | Conclusions on the food/feed safety assessment

The GMO Panel conducted a comprehensive assessment of oilseed rape LBFLFK under a full-scope application, following the current risk assessment approach in accordance with Regulation (EU) No 503/2013.[3] The applicant provided information throughout the assessment process, including answers to 17 clock stop letters and unsolicited data (spontaneous information).

Protein safety assessment

The GMO Panel concludes that, based on the information provided by the applicant, the safety of the elongases and desaturases newly expressed in oilseed rape LBFLFK cannot be established. The AHAS protein, also newly expressed in oilseed rape LBFLFK, does not raise safety concerns for human and animal health.

Fatty acid assessment

Human nutrition assessment of RBD oil produced from oilseed rape LBFLFK

The GMO Panel cannot conclude on the full-scope of potential food uses of oilseed rape LBFLFK. However, based on the outcome of the comparative compositional analysis and the nutritional assessment, the GMO Panel concludes that the consumption of RBD LBFLFK oil is safe and does not represent any nutritional concern in humans under the conditions of use proposed by the applicant and considered during the pre-market risk assessment (see Appendix C). If these conditions of use change (foods and maximum fatty acid concentrations in these foods), the current nutritional assessment will have to be revisited.

A PMM plan, in line with Article 6(5)(e) of Regulation (EC) No 1829/2003 and as indicated in Regulation (EU) No 503/2013,[3] is recommended to confirm the predicted consumption and the application of conditions of uses for the RBD LBFLFK oil in food considered during the pre-market risk assessment.

Animal nutrition assessment of oil produced from oilseed rape LBFLFK

Based on the results of the comparative compositional analysis, the nutritional assessment and the available feeding studies in aquatic species, the GMO Panel concludes that the safety and nutritional adequacy of the oil and full-fat seeds derived from oilseed rape LBFLFK for its use in terrestrial farmed and companion animals and in aquaculture cannot be established. Consequently, it is also not in a position to provide comments on the post-market monitoring of the GM feed.

3.6 | Environmental risk assessment and monitoring plan[29]

3.6.1 | Environmental risk assessment

The scope of this application is for food and feed uses, import and processing, and excludes cultivation. Processed products from oilseed rape (i.e. defatted meal, containing NEPs and oil, not containing NEPs) can be used as feed for farmed aquatic and terrestrial animals. Therefore the environmental risk assessment (ERA) mainly takes into account: (1) the deliberate or accidental release into the receiving environments of processed and unprocessed GM material, including spillage of viable seeds during transportation and/or processing; and (2) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed with GM material and of microorganisms present in all environments exposed to manure and faeces of these animals (EFSA GMO Panel, 2010a).

3.6.1.1 | *Persistence and invasiveness of the GM plant*

Oilseed rape (*Brassica napus* AACC) 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 (Beckie & Warwick, 2010; Belter, 2016; D'Hertefeldt et al., 2008; Gruber et al., 2008; Lutman et al., 2003, 2005, 2008; Messéan et al., 2007; Peltonen-Sainio & Jauhiainen, 2014) 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. Bailleul et al., 2016; Bauer-Panskus et al., 2013; Busi & Powles, 2016; COGEM, 2013; Devos et al., 2012; Franzaring et al., 2016; Hecht et al., 2014; Katsuta et al., 2015; Nishizawa et al., 2016; Pandolfo et al., 2016; Schulze et al., 2014). Oilseed rape is generally regarded as an opportunistic species, which can take advantage of disturbed sites (e.g. mowed areas) 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 (Busi & Powles, 2016; COGEM, 2013). Once established in competition-free germination sites, feral populations decline over a period of years (Banks, 2014; Busi & Powles, 2016; Crawley & Brown, 1995, 2004; Knispel et al., 2008; Squire et al., 2011). However, if habitats are disturbed on a regular basis, then feral populations can persist for longer periods (Claessen et al., 2005a; Claessen et al., 2005b; Garnier et al., 2006) and can have the characteristics of a weed or ruderal (Banks, 2014). 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 (Bailleul et al., 2016; Banks, 2014; Pivard et al., 2008, b).

The applicant provided an additional study[30] to investigate how the genetic modification of oilseed rape LBFLFK might alter its seed germination and secondary dormancy. However, the study is of limited value to draw conclusions on the potential risk of invasiveness and persistence of oilseed rape LBFLFK because of the highly controlled laboratory conditions under which the materials were tested.

[29]Dossier: Part II – Section 5 and 6; additional information: 3/4/2024; spontaneous information: 19/3/2021.

[30]Study 201703-008R Germination and Fatty Acid Profile of Stored LBFLFK Seeds of EPA + DHA Canola.

It is unlikely that the intended traits of oilseed rape LBFLFK and the observed reduction in germination of harvested seeds (F_2 generation) will provide a selective advantage to oilseed rape LBFLFK plants, except when they are exposed to imidazolinone-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 LBFLFK to overcome other biological and abiotic factors (described above) limiting plant's persistence and invasiveness.

In conclusion, the GMO Panel considers it very unlikely that oilseed rape LBFLFK will lead to an increased ability to survive and establish feral populations under European environmental conditions in case of accidental release into the environment of viable oilseed rape LBFLFK 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 HGT of DNA, or through vertical gene flow via cross-pollination from feral plants originating from spilled seeds.

3.6.1.2.1 | *Plant-to-microorganism gene transfer*

Genomic DNA can be a component of food and feed products derived from oilseed rape LBFLFK. 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, 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, chromosome-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 (EFSA, 2009; Hulter & Wackernagel, 2008). Regardless 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 revealed for event LBFLFK sufficient sequence identity with two t-OCS originated from *Agrobacterium tumefaciens* that are the terminators of the octopine synthase gene (t-OCS) (EFSA, 2017b). The sequence between the t-OCS of the plant insert has a length of about 30 Kbp and carries plant codon-optimised versions of several microbial genes and a gene of plant origin (see Section 3.3.1). The GMO panel therefore considered that the sequence identity of these two t-OCS sequences has the potential to facilitate double HR with Ti plasmids of environmental *A. tumefaciens* strains, resulting in the insertion of a large DNA fragment (about 30 Kbp). The large size of this insert, however, decreases the likelihood for HGT. Recipients receiving a Ti plasmid with such a large DNA insert and without a selective advantage, would also most likely be affected in their fitness because of the additional burden of replicating non-functional DNA in their cells during growth.

In summary, the assessment of a potential HGT from oilseed rape LBFLFK to bacteria did not raise any safety concern.

3.6.1.2.2 | *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, 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/reported to be rare with *B. nigra*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapis arvensis* (reviewed by Liu et al., 2013; FitzJohn et al., 2007; Devos et al., 2009; Tang et al., 2018; Laforest et al., 2022). Of significance, transgene introgression has only been confirmed under field conditions for *B. rapa* (Hansen et al., 2001, 2003; Jørgensen, 2007; Jørgensen et al., 2004; Laforest et al., 2022; Norris et al., 2004; Warwick et al., 2003, 2008). The introgression from *B. napus* and stabilisation in the *B. rapa* genome through a series of backcrosses reported under cultivation conditions in Laforest et al. (2022) highlights the impact of inappropriate weed management in an agricultural setting. 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 & 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 (Elling et al., 2009; Jørgensen et al., 2004; Laforest et al., 2022; Norris et al., 2004; Warwick et al., 2008), or at ports, along roadsides and riverbanks (Aono et al., 2006, 2011; Elling et al., 2009; Katsuta et al., 2015; Luijten et al., 2015; Saji et al., 2005; Yoshimura et al., 2006).

The GMO Panel does not consider the occurrence of feral oilseed rape LBFLFK plants, pollen dispersal and consequent cross-pollination as environmental harm in itself, as there is no evidence that the traits will enhance the vertical gene flow potential, or fitness, persistence or invasiveness of feral oilseed rape LBFLFK, or cross-compatible plants such as hybridising wild relatives. However, when exposed to imidazolinone-containing herbicides, occasional cross-compatible plants that acquired the herbicide tolerance traits through vertical gene flow are likely to exhibit a selective advantage, which may lead to their increased abundance. The likelihood of such an event happening 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 traits 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 LBFLFK 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-DE-2019-157 (no cultivation) and the absence of target organisms into account, potential interactions of occasional feral oilseed rape LBFLFK 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*

The environmental risk assessment considers potential effects of the GM plant on populations of non-target organisms (NTOs), defined as all those species directly or indirectly exposed to processed and unprocessed oilseed rape LBFLFK.

Regarding the terrestrial environment, NTOs can be exposed to processed feed products containing oil or NEPs derived from oilseed rape LBFLFK, but also to seed import spills and plants arising from them. Given that terrestrial plants do not typically produce the LC-PUFAs EPA and DHA, oilseed rape LBFLFK would be the first terrestrial source of these compounds for NTOs (Gladyshev et al., 2013; Hixson et al., 2016). A very limited number of studies have addressed the potential effects of EPA and DHA on terrestrial herbivores, whereas no reports have been published on their potential indirect effects on higher trophic levels (Colombo et al., 2018, 2020; Hixson et al., 2016; Lehtovaara et al., 2017). The GMO Panel acknowledges that the scarcity of research in this area creates uncertainty.

Based on the information provided by the applicant, the GMO Panel concludes that the safety of the oil produced from oilseed rape LBFLFK and of the related NEPs expressed in this GM crop for terrestrial and aquatic animals cannot be established (See Section 3.5.6). In the terrestrial environment exposure to spilled processed and unprocessed material and plants arising from spilled seeds is expected to be very limited; therefore the GMO Panel concludes that there are no environmental safety concerns for terrestrial NTOs. According to the intended uses declared by the applicant, exposure in the aquatic environment is expected to be high. Given the inconclusiveness of the assessment of the safety of feed products containing oil or NEPs produced by oilseed rape LBFLFK and the significant exposure expected in the aquatic environment in a full replacement scenario, the GMO Panel cannot conclude on the safety of oilseed rape LBFLFK for NTOs in the aquatic environment.

3.6.1.5 | *Interactions with biogeochemical cycles*

Biogeochemical cycles encompass the microbiologically mediated movement, transformation and storage of carbon, nitrogen and other compounds that are considered here for the receiving environments. Based on the information provided by the applicant, the GMO Panel concludes that the safety of the NEPs expressed in oilseed rape LBFLFK cannot be established (See Section 3.5.1).

In the terrestrial environment, exposure to spilled GM oilseed rape material or occasional feral GM oilseed rape plants arising from spilled oilseed rape LBFLFK seeds will be low, whereas higher exposure to manure and faeces of animals fed with oilseed rape LBFLFK material cannot be excluded. However, ingested proteins are typically degraded before entering the environment through manure and faeces of animals fed with GM oilseed rape (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernandez et al., 2018; Vanbruchem et al., 1985). Overall, the GMO Panel concludes that there are no environmental safety concerns for biogeochemical cycles in the terrestrial environment.

Exposure to processed oilseed rape LBFLFK in the aquatic environment is expected to be high. The GMO Panel cannot conclude on whether the potential effects of oilseed rape LBFLFK on biogeochemical cycles in the aquatic environment raise any environmental safety concerns, due to the lack of data on the safety of the NEPs and oil and the expected high exposure in this environment.

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).

The PMEM plan proposed by the applicant for oilseed rape LBFLFK consists only of general surveillance and it 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., 2009). The applicant proposes to submit a PMEM report on an annual basis for the duration of the authorisation period.

The GMO Panel is unable to conclude on the environmental safety of oilseed rape LBFLFK oil and protein containing products for all areas of concern and environments (See Sections 3.6.1.4 and 3.6.1.5). Therefore, the GMO Panel cannot conclude whether the PMEM plan proposed by the applicant is adequate for the intended uses of oilseed rape LBFLFK.

Under the assumption of a limited authorisation to oil for human consumption (see Section 3.5.6), PMEM would not be necessary as no viable material would be imported.

3.6.2.1 | *Conclusion of the environmental risk assessment and monitoring plan*

The GMO Panel concludes that it is unlikely that oilseed rape LBFLFK will have an increased ability to persist under European environmental conditions or as a consequence of the spread of genes from oilseed rape LBFLFK. The assessment of potential HGT from oilseed rape LBFLFK to bacteria does not raise any safety concerns. Based on the different levels of environmental exposure expected, the GMO Panel concludes that there are no environmental safety concerns for terrestrial NTOs or biogeochemical cycles in this environment, whereas the GMO Panel cannot conclude on the safety of oilseed rape LBFLFK to NTOs or biogeochemical cycles in the aquatic environment.

Since the GMO Panel could not conclude the environmental risk assessment for all areas of concern and environments, it is not possible to conclude on the adequacy of the PMEM plan proposed by the applicant for oilseed rape LBFLFK.

4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of oilseed rape LBFLFK. The scope of the application is for food and feed uses, import and processing of oilseed rape LBFLFK submitted within the framework of Regulation (EC) No 1829/2003.

The molecular characterisation data establish that oilseed rape LBFLFK contains 2 inserts (Insert 1 and Insert 2), not genetically linked, each consisting of a single copy of the T-DNA originating from the same transformation vector, which contains 13 expression cassettes (coding for 11 unique proteins). The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and complies with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the NEPs, the sequences corresponding to ORFs within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise safety concerns. The stability of the inserted DNA and of the introduced trait conferring herbicide tolerance and an altered fatty acid composition was confirmed over several generations. The methodology used to quantify the levels of the NEPs is considered adequate. As most of the NEPs could not be produced in a heterologous expression system in the amounts and purity sufficient for the protein characterisation, the description of biochemical, structural and functional properties of the NEPs was performed with a membrane fraction purified from crude extracts of developing embryos that were isolated from immature seeds of oilseed rape LBFLFK (plant-produced proteins, PPP). The activities of all the introduced elongases and desaturases for the intended substrates were demonstrated upon expression in yeast. However, several protein characterisation assays failed for a number of NEPs in PPP. These include protein detection using LC–MS/MS and/or western blotting that was not successful for D6E(*Pp*) and O3D(*Pi*), and absence of detectable enzyme activity specific for D6D(*Ot*), D6E (*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), O3D(*Pi*), D4D(*Pl*) and D4D(*Tc*). Moreover, based on the provided data, partial glycosylation of D12D(*Ps*), D6D(*Ot*), D6E(*Pp*), D5D(*Tc*), O3D(*Pir*), D5E(*Ot*) and D4D(*Tc*) cannot be excluded. Due to failure of some of the analyses, the GMO panel cannot conclude that a membrane fraction purified from crude extracts of developing embryos is an appropriate source of NEP for the safety studies.

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 differences identified in agronomic and phenotypic characteristics between oilseed rape LBFLFK and the non-GM comparator needed further assessment except

for germination of harvested seeds, which underwent additional evaluation and was found not to raise environmental concerns.

The GMO Panel was unable to conclude on the safety of oilseed rape LBFLFK when considering the full-scope of the application. The GMO Panel conducted a protein safety assessment of the newly expressed proteins in oilseed rape LBFLFK based on the information provided by the applicant. The GMO Panel concludes that the safety of the elongases and desaturases newly expressed in oilseed rape LBFLFK cannot be established. The AHAS protein, also newly expressed in oilseed rape LBFLFK, does not raise safety concerns for human and animal health. In the case of the human nutritional assessment, the applicant provided detailed information on the intended uses of the RBD oil, and submitted a list of food products with a predefined composition of relevant fatty acids (see Appendix C). Based on this information, the GMO Panel concludes that the consumption of RBD LBFLFK oil is safe and does not represent any nutritional concern in humans under the conditions of use proposed by the applicant and considered during the pre-market risk assessment (see Appendix C). If these conditions of use change (foods and maximum fatty acid concentrations in these foods), the current nutritional assessment will have to be revisited.

A PMM plan, in line with Article 6(5)(e) of Regulation (EC) No 1829/2003 and as indicated in Regulation (EU) No 503/2013,[3] is recommended to confirm the predicted consumption and the application of conditions of uses for the RBD LBFLFK oil in food considered during the pre-market risk assessment. The GMO Panel considers that the proposed labelling is not scientifically correct as it does not fully characterise the compositional changes in the GM crop. Therefore, the labelling should be adjusted to be scientifically correct. Furthermore, considering the potential safety concerns identified for frying or other high-temperature uses of oils rich in PUFAs, appropriate labelling of specific food uses (e.g. margarine, butter, oil; see Appendix C for details) could help ensure that these foods are not used for high-temperature applications.

In animals, based on the results of the comparative compositional analysis, the nutritional assessment and the available feeding studies in aquatic species, the GMO Panel concludes that the safety and nutritional adequacy of the oil and full-fat seeds derived from oilseed rape LBFLFK for its use in terrestrial farmed and companion animals and in aquaculture cannot be established. Consequently, it is also not in a position to provide comments on the post-market monitoring of the GM feed.

The GMO Panel did not identify any environmental concerns related to increased persistence and invasiveness or to any potential for gene transfer from oilseed rape LBFLFK. Likewise, the GMO Panel concludes that oilseed rape LBFLFK poses no risks for terrestrial NTOs or biogeochemical cycles in this environment, whereas the GMO Panel cannot conclude on the safety of oilseed rape LBFLFK to NTOs or biogeochemical cycles in the aquatic environment. Owing to the inconclusiveness of the environmental risk assessment for some areas of concern and environments, the GMO Panel is unable to conclude on the adequacy of the PMEM plan proposed by the applicant.

The GMO Panel cannot conclude on the safety of oilseed rape LBFLFK when considering the full-scope of the application. On the other hand, the GMO Panel concludes that the consumption of RBD LBFLFK oil is safe and does not represent any nutritional concern for humans under the conditions of use proposed by the applicant and considered during the pre-market risk assessment.

5 | DOCUMENTATION AS PROVIDED TO EFSA

- Letter from the Competent Authority of Germany received on 20 June 2019 concerning a request for authorization of import to the European Union of genetically modified canola LBFLFK, submitted in accordance with Regulation (EC) No 1829/2003 by BASF Agricultural Solutions Belgium NV on behalf of BASF Plant Science Company GmbH (EFSA Ref. EFSA-GMO-DE-2019-157; EFSA-Q-2019-00394).
- The application was made valid on 27 November 2019.
- Additional information (1) was requested on 3 December 2019.
- Additional information (1) was received on 4 February 2020.
- Additional information (2) was requested on 26 February 2020.
- Additional information (2) was received on 20 May 2020.
- Additional information (3) was requested on 5 May 2020.
- Additional information (3) was received on 2 July 2020 partial; 30 September 2020 complete.
- Additional information (4) was requested on 6 October 2020.
- Additional information (4) was received on 8 December 2020.
- Additional information (5) was requested on 23 December 2020.
- Additional information (5) was received on 24 February 2021 partial; 28 April 2021 complete.
- Additional information (6) was requested on 9 February 2021.
- Additional information (6) was received on 31 March 2021.
- Additional information (7) was requested on 10 May 2021.
- Additional information (7) was received on 23 August 2021.
- Additional information (8) was requested on 12 July 2021.
- Additional information (8) was received on 24 September 2021 partial; 12 January 2022 complete.
- Additional information (9) was requested on 3 March 2022.
- Additional information (9) was received on 3 May 2022.

- Additional information (10) was requested on 20 May 2022.
- Additional information (10) was received on 30 September 2022.
- Additional information (11) was requested on 7 October 2022.
- Additional information (11) was received on 6 April 2023.
- Additional information (12) was requested on 16 March 2023.
- Additional information (12) was received on 17 July 2023.
- Additional information (13) was requested on 18 July 2023.
- Additional information (13) was received on 30 November 2023.
- Additional information (14) was requested on 5 December 2023.
- Additional information (14) was received on 5 February 2024 partial; 3 April 2024 complete.
- Additional information (15) was requested on 12 February 2024.
- Additional information (15) was received on 3 May 2024.
- Additional information (16) was requested on 13 May 2024.
- Additional information (16) was received on 10 June 2024.
- Additional information (17) was requested on 6 June 2024.
- Additional information (17) was received on 3 October 2024.
- Spontaneous information was received on 19 March 2021; 11 October 2022; 31 July 2023; 31 July 2023; 15 December 2023 and 12 June 2025.

ABBREVIATIONS

ACP	acyl carrier protein
ARA	arachidonic acid
ANOVA	analysis of variance
bp	base pair
bw	body weight
CoA	coenzyme A
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DPA	clupanodonic acid
dw	dry weight
ELO	elongase
EPA	eicosapentaenoic acid
ERA	environmental risk assessment
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDA	Food and Drug Administration
fw	fresh weight
GLA	γ-linolenic acid
GM	genetically modified
GMO	genetically modified organisms
HGT	horizontal gene transfer
HoSU	history of safe use for consumption
HR	homologous recombination
kDa	kilodalton
KS	ketoacyl synthase
LB	left border
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LC-PUFA	long-chain polyunsaturated fatty acid
LOD	limit of detection
LOQ	limit of quantification
NEP	newly expressed protein
NGS	next-generation sequencing
NTO	non-target organism
OECD	Organisation for Economic Co-operation and Development
OLA	oleic acid
ORF	open reading frame
PCR	polymerase chain reaction
PMEM	post-market environmental monitoring
PMM	post-market monitoring
PPP	plant-produced proteins
PUFA	polyunsaturated fatty acid
RB	right border

RBD	refined, bleached and deodorised
SDA	stearidonic acid
T-DNA	transfer-deoxyribonucleic acid
t-OCS	octopine synthase gene terminator
TFA	trans-fatty acid
UL	upper intake levels
UTR	untranslated region

ACKNOWLEDGEMENTS

The Panel wishes to thank the members of the Working Groups on Molecular characterisation, Food and Feed Safety Assessment and Working Group on Comparative Analysis and Environmental Risk Assessment for the preparatory work on this scientific output, and the EFSA staff members Aina Belén Gil González, Paschalina Grammatikou, Yustina Olshevska Grigorov and Konstantinos Paraskevopoulos for the support provided to this scientific output.

REQUESTOR

Competent Authority of Germany

QUESTION NUMBER

EFSA-Q-2019-00394

PANEL MEMBERS

Josep Casacuberta, Francisco Barro, Albert Braeuning, Ruud de Maagd, Michelle M. Epstein, Thomas Frenzel, Jean-Luc Gallois, Frits Koning, Antoine Messéan, F. Javier Moreno, Fabien Nogué, Giovanni Savoini, Alan H. Schulman, Christoph Tebbe and Eve Veromann.

REFERENCES

Alasalvar, C., Taylor, K. D. A., Zubcov, E., Shahidi, F., & Alexis, M. (2002). Differentiation of cultured and wild sea bass (Dicentrarchus labrax): total lipid content, fatty acid and trace mineral composition. *Food Chemistry*, *79*(2), 145–150. https://doi.org/10.1016/S0308-8146(02)00122-X

Andre, C., Buesen, R., Riffle, B., Wandelt, C., Sottosanto, J. B., Marxfeld, H., Strauss, V., van Ravenzwaay, B., & Lipscomb, E. A. (2019). Safety assessment of EPA + DHA canola oil by fatty acid profile comparison to various edible oils and fat-containing foods and a 28-day repeated dose toxicity study in rats. *Food and Chemical Toxicology*, *124*, 168–181. https://doi.org/10.1016/j.fct.2018.11.042

Aono, M., Wakiyama, S., Nagatsu, M., Kaneko, Y., Nishizawa, T., Nakajima, N., Tamaoki, M., Kubo, A., & Saji, H. (2011). Seeds of a possible natural hybrid between herbicide-resistant Brassica napus and Brassica rapa detected on a riverbank in Japan. *GM Crops*, *2*(3), 201–210. https://doi.org/10.4161/gmcr.2.3.18931

Aono, M., Wakiyama, S., Nagatsu, M., Nakajima, N., Tamaoki, M., Kubo, A., & Saji, H. (2006). Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. *Environmental Biosafety Research*, *5*(2), 77–87. https://doi.org/10.1051/ebr:2006017

Bailleul, D., Ollier, S., & Lecomte, J. (2016). Genetic diversity of oilseed rape fields and feral populations in the context of coexistence with GM crops. *PLoS One*, *11*(6), e0158403. https://doi.org/10.1371/journal.pone.0158403

Banks, G. (2014). Feral oilseed rape populations within a scottish landscape: Implications for GM coexistence and environmental risk assessment. University of Dundee. https://discovery.dundee.ac.uk/en/studentTheses/feral-oilseed-rape-populations-within-a-scottish-landscape.

Bauer-Panskus, A., Breckling, B., Hamberger, S., & Then, C. (2013). Cultivation-independent establishment of genetically engineered plants in natural populations: Current evidence and implications for EU regulation. *Environmental Sciences Europe*, *25*(1), 34. https://doi.org/10.1186/2190-4715-25-34

Beccaria, M., Costa, R., Sullini, G., Grasso, E., Cacciola, F., Dugo, P., & Mondello, L. (2015). Determination of the triacylglycerol fraction in fish oil by comprehensive liquid chromatography techniques with the support of gas chromatography and mass spectrometry data. *Analytical and Bioanalytical Chemistry*, *407*(17), 5211–5225. https://doi.org/10.1007/s00216-015-8718-y

Beckie, H. J., & Warwick, S. I. (2010). Persistence of an oilseed rape transgene in the environment. *Crop Protection*, *29*(5), 509–512. https://doi.org/10.1016/j.cropro.2009.11.013

Belter, A. (2016). Long-term monitoring of field trial sites with genetically modified oilseed rape (Brassica napus L.) in Saxony-Anhalt, Germany. Fifteen Years Persistence to Date but No Spatial Dispersion. *Genes*, *7*(1), 3. https://doi.org/10.3390/genes7010003

Busi, R., & Powles, S. B. (2016). Transgenic glyphosate-resistant canola (Brassica napus) can persist outside agricultural fields in Australia. *Agriculture Ecosystems & Environment*, *220*, 28–34. https://doi.org/10.1016/j.agee.2015.12.028

Çelik, M., Türeli, C., Çelik, M., Yanar, Y., Erdem, Ü., & Küçükgülmez, A. (2004). Fatty acid composition of the blue crab (Callinectes sapidus Rathbun, 1896) in the north eastern Mediterranean. *Food Chemistry*, *88*(2), 271–273. https://doi.org/10.1016/j.foodchem.2004.01.038

Claessen, D., Gilligan, C. A., Lutman, P. J. W., & van den Bosch, F. (2005). Which traits promote persistence of feral GM crops? Part 1: Implications of environmental stochasticity. *Oikos*, *110*(1), 20–29. https://doi.org/10.1111/j.0030-1299.2005.13667.x

Claessen, D., Gilligan, C. A., & van den Bosch, F. (2005). Which traits promote persistence of feral GM crops? Part 2: Implications of metapopulation structure. *Oikos*, *110*(1), 30–42. https://doi.org/10.1111/j.0030-1299.2005.13668.x

Codex Alimentarius Comission. (2009). Foods derived from modern biotechnology (second Edition ed.). FAO and WHO. https://www.fao.org/3/a1554e/a1554e00.pdf

COGEM. (2013). Genetically modified oilseed rape (Brassica napus) (COGEM Advisory report, Issue). https://cogem.net/app/uploads/2019/07/130402-01-Advisory-report-Genetically-modified-oilseed-rape.pdf.

Colombo, S. M., Campbell, L. G., Murphy, E. J., Martin, S. L., & Arts, M. T. (2018). Potential for novel production of omega-3 long-chain fatty acids by genetically engineered oilseed plants to alter terrestrial ecosystem dynamics. *Agricultural Systems*, *164*, 31–37. https://doi.org/10.1016/j.agsy.2018.03.004

Colombo, S. M., Shukla, K., Campbell, L. G., Tsimbaliouk, A., & Arts, M. T. (2020). Dietary eicosapentaenoic acid and docosahexaenoic acid are linearly retained by common insect crop pests (cabbage looper and bertha armyworm) and alter insect biomass. *Physiological Entomology*, *45*(1), 38–49. https://doi.org/10.1111/phen.12314

Costa, R., Albergamo, A., Piparo, M., Zaccone, G., Capillo, G., Manganaro, A., Dugo, P., & Mondello, L. (2017). Multidimensional gas chromatographic techniques applied to the analysis of lipids from wild-caught and farmed marine species. *European Journal of Lipid Science and Technology*, *119*(2), 1600043.

Crawley, M. J., & Brown, S. L. (1995). Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society B: Biological Sciences*, *259*(1354), 49–54. https://doi.org/10.1098/rspb.1995.0008

Crawley, M. J., & Brown, S. L. (2004). Spatially structured population dynamics in feral oilseed rape. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1551), 1909–1916. https://doi.org/10.1098/rspb.2004.2814

Crawley, M. J., Brown, S. L., Hails, R. S., Kohn, D. D., & Rees, M. (2001). Biotechnology - transgenic crops in natural habitats. *Nature*, *409*(6821), 682–683. https://doi.org/10.1038/35055621

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D., & Buxton, J. (1993). Ecology of transgenic oilseed rape in natural habitats. *Nature*, *363*(6430), 620–623. https://doi.org/10.1038/363620a0

Cui, W., Liang, Y., Tian, W., Ji, M., & Ma, X. (2016). Regulating effect of beta-ketoacyl synthase domain of fatty acid synthase on fatty acyl chain length in de novo fatty acid synthesis. *Biochimica et Biophysica Acta*, *1861*(3), 149–155. https://doi.org/10.1016/j.bbalip.2015.12.002

de Jong, T. J., & Rong, J. (2013). Crop to wild gene flow: Does more sophisticated research provide better risk assessment? *Environmental Science & Policy*, *27*, 135–140. https://doi.org/10.1016/j.envsci.2012.12.002

Denic, V., & Weissman, J. S. (2007). A molecular caliper mechanism for determining very long-chain fatty acid length. *Cell*, *130*(4), 663–677. https://doi.org/10.1016/j.cell.2007.06.031

Devle, H., Vetti, I., Naess-Andresen, C. F., Rukke, E.-O., Vegarud, G., & Ekeberg, D. (2012). A comparative study of fatty acid profiles in ruminant and non-ruminant milk. *European Journal of Lipid Science and Technology*, *114*(9), 1036–1043. https://doi.org/10.1002/ejlt.201100333

Devos, Y., De Schrijver, A., & Reheul, D. (2009). Quantifying the introgressive hybridisation propensity between transgenic oilseed rape and its wild/weedy relatives. *Environmental Monitoring and Assessment*, *149*(1–4), 303–322. https://doi.org/10.1007/s10661-008-0204-y

Devos, Y., Hails, R. S., Messéan, A., Perry, J. N., & Squire, G. R. (2012). Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? *Transgenic Research*, *21*(1), 1–21. https://doi.org/10.1007/s11248-011-9515-9

D'Hertefeldt, T., Jorgensen, R. B., & Pettersson, L. B. (2008). Long-term persistence of GM oilseed rape in the seedbank. *Biology Letters*, *4*(3), 314–317. https://doi.org/10.1098/rsbl.2008.0123

Eastham, K. A. S. J. (2002). Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer (28). (Environmental Issue Report, Issue). http://www.eea.europa.eu/publications/environmental_issue_report_2002_28

EFSA (European Food Safety Authority). (2009). Consolidated presentation of the joint scientific opinion of the GMO and BIOHAZ panels on the "use of antibiotic resistance genes as marker genes in genetically modified plants" and the scientific opinion of the GMO panel on "consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". *EFSA Journal*, *7*(6), 1108. https://doi.org/10.2903/j.efsa.2009.1108

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

EFSA (European Food Safety Authority). (2014). Explanatory statement for the applicability of the guidance of the EFSA scientific committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. *EFSA Journal*, *12*(10), 3871. https://doi.org/10.2903/j.efsa.2014.3871

EFSA (European Food Safety Authority). (2015). The food classification and description system FoodEx2 (revision 2). *EFSA Supporting Publications*, EN-804. https://doi.org/10.2903/sp.efsa.2015.EN-804

EFSA (European Food Safety Authority). (2017a). Explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. *EFSA Supporting Publications*, *14*(7), 1273E. https://doi.org/10.2903/sp.efsa.2017.EN-1273

EFSA (European Food Safety Authority). (2017b). Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. *EFSA Supporting Publications*, *14*(4), 1207E. https://doi.org/10.2903/sp.efsa.2017.EN-1207

EFSA (European Food Safety Authority). (2018). Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation. *EFSA Supporting Publications*, *15*(8), 1466E. https://doi.org/10.2903/sp.efsa.2018.EN-1466

EFSA (European Food Safety Authority). (2019a). Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. *EFSA Supporting Publications*, *16*(4), 1614E. https://doi.org/10.2903/sp.efsa.2019.EN-1614

EFSA (European Food Safety Authority). (2019b). Statement on the human dietary exposure assessment to newly expressed proteins in GM foods. *EFSA Journal*, *17*(7), e05802. https://doi.org/10.2903/j.efsa.2019.5802

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010a). Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal*, *8*(11), 1879. https://doi.org/10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010b). Statistical considerations for the safety evaluation of GMOs. *EFSA Journal*, *8*(2), 1250. https://doi.org/10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011a). Guidance for risk assessment of food and feed from genetically modified plants. *EFSA Journal*, *9*(5), 2150. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011b). Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. *EFSA Journal*, *9*(8), 2316. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2014). Scientific opinion on application (EFSA-GMO-NL-2009-64) for the placing on the market of herbicide-tolerant genetically modified soybean BPS-CV127-9 for food and feed uses, import and processing under regulation (EC) No 1829/2003 from BASF Plant science. *EFSA Journal*, *12*(1), 3505. https://doi.org/10.2903/j.efsa.2014.3505

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2015). Guidance on the agronomic and phenotypic characterisation of genetically modified plants. *EFSA Journal*, *13*(6), 4128. https://doi.org/10.2903/j.efsa.2015.4128

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Nogué, F., Naegeli, H., Birch, A. N., De Schrijver, A., Gralak, M. A., Guerche, P., Manachini, B., Messéan, A., Nielsen, E. E., Robaglia, C., Rostoks, N., Sweet, J., Tebbe, C., Visioli, F., Wal, J.-M., Moxon, S., Schneeberger, K., … Jones, H. (2018). Technical note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants. *EFSA Journal*, *16*(7), 5345. https://doi.org/10.2903/j.efsa.2018.5345

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Mullins, E., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., & Fernandez Dumont, A. (2021). Statement on in vitro protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants. *EFSA Journal*, *19*(1), 6350. https://doi.org/10.2903/j.efsa.2021.6350

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., George Firbank, L., Guerche, P., Hejatko, J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Fernandez Dumont, A., & Moreno, F. J. (2022a). Scientific opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. *EFSA Journal*, *20*(1), 7044. https://doi.org/10.2903/j.efsa.2022.7044

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Nogue, F., Rostoks, N., Sanchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Casacuberta, J., Zurbriggen, M. D., Fernandez, A., … Naegeli, H. (2022b). Evaluation of existing guidelines for their adequacy for the food and feed risk assessment of genetically modified plants obtained through synthetic biology. *EFSA Journal*, *20*(7), 7410. https://doi.org/10.2903/j.efsa.2022.7410

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Barro, F., Braeuning, A., de Maagd, R., Epstein, M. M., Frenzel, T., Gallois, J. L., Koning, F., Messéan, A., Moreno, F. J., Nogué, F., Savoini, G., Schulman, A. H., Tebbe, C., Veromann, E., Ardizzone, M., Belmonte, L., & Bonatti, M. (2025). Current practice, challenges and future opportunities in the safety assessment of newly expressed proteins in genetically modified plants. *EFSA Journal*, *23*(8), 9568. https://doi.org/10.2903/j.efsa.2025.9568

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies). (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461. https://doi.org/10.2903/j.efsa.2010.1461

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition, and Allergies). (2012). Scientific opinion related to the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). *EFSA Journal*, *10*(7), 2815. https://doi.org/10.2903/j.efsa.2012.2815

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2013). Scientific opinion on the safety of "rapeseed protein isolate" as a novel food ingredient. *EFSA Journal*, *11*(10), 3420. https://doi.org/10.2903/j.efsa.2013.3420

EFSA NDA Panel (EFSA Panel on Nutrition, Novel Foods and Food Allergens). (2020). Scientific opinion on the safety of rapeseed powder from Brassica rapa L. and Brassica napus L. as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal*, *18*(7), 6197. https://doi.org/10.2903/j.efsa.2020.6197

EFSA Scientific Committee. (2011). Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. *EFSA Journal*, *9*(12), 2438. https://doi.org/10.2903/j.efsa.2011.2438

EFSA Scientific Committee. (2012). Guidance on selected default values to be used by the EFSA scientific committee, scientific panels and units in the absence of actual measured data. *EFSA Journal*, *10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

Elling, B., Neuffer, B., & Bleeker, W. (2009). Sources of genetic diversity in feral oilseed rape (Brassica napus) populations. *Basic and Applied Ecology*, *10*(6), 544–553. https://doi.org/10.1016/j.baae.2009.01.005

Ellstrand, N. C., Prentice, H. C., & Hancock, J. F. (1999). Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics*, *30*, 539–563. https://doi.org/10.1146/annurev.ecolsys.30.1.539

FAO (Food and Agriculture Organization). (2010). Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food and Nutrition Paper*, *91*, 1–166.

FitzJohn, R. G., Armstrong, T. T., Newstrom-Lloyd, L. E., Wilton, A. D., & Cochrane, M. (2007). Hybridisation within brassica and allied genera: evaluation of potential for transgene escape. *Euphytica*, *158*(1–2), 209–230. https://doi.org/10.1007/s10681-007-9444-0

Foseid, L., Devle, H., Stenstrøm, Y., Naess-Andresen, C. F., & Ekeberg, D. (2017). Fatty acid profiles of stipe and blade from the Norwegian brown macroalgae Laminaria hyperborea with special reference to acyl glycerides, polar lipids, and free fatty acids. *Journal of Lipids*, *2017*(1), 1029702. https://doi.org/10.1155/2017/1029702

Franzaring, J., Wedlich, K., Fangmeier, A., Eckert, S., Zipperle, J., Krah-Jentgens, I., Hünig, C., & Züghart, W. (2016). Exploratory study on the presence of GM oilseed rape near German oil mills. *Environmental Science and Pollution Research*, *23*(22), 300–307. https://doi.org/10.1007/s11356-016-7735-5

Garnier, A., Deville, A., & Lecomte, J. (2006). Stochastic modelling of feral plant populations with seed immigration and road verge management. *Ecological Modelling*, *197*(3–4), 373–382. https://doi.org/10.1016/j.ecolmodel.2006.03.031

Garnier, A., Darmency, H., Tricault, Y., Chèvre, A. M., & Lecomte, J. (2014). A stochastic cellular model with uncertainty analysis to assess the risk of transgene invasion after crop-wild hybridization: Oilseed rape and wild radish as a case study. *Ecological Modelling*, *276*, 85–94. https://doi.org/10.1016/j.ecolmodel.2014.01.004

Ghioni, C., Porter, A. E. A., Taylor, G. W., & Tocher, D. R. (2002). Metabolism of 18:4n-3 (stearidonic acid) and 20:4n-3 in salmonid cells in culture and inhibition of the production of prostaglandin F2α (PGF2α) from 20:4n-6 (arachidonic acid). *Fish Physiology and Biochemistry*, *27*(1–2), 81–96. https://doi.org/10.1023/B:Fish.0000021866.78048.45

Gladyshev, M. I., Sushchik, N. N., & Makhutova, O. N. (2013). Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins & Other Lipid Mediators*, *107*, 117–126. https://doi.org/10.1016/j.prostaglandins.2013.03.002

Grigorakis, K., Giogios, I., Vasilaki, A., & Nengas, I. (2010). Effect of the fish oil, oxidation status and of heat treatment temperature on the volatile compounds of the produced fish feeds. *Animal Feed Science and Technology*, *158*(1–2), 73–84. https://doi.org/10.1016/j.anifeedsci.2010.03.012

Grootveld, M. (2022). Evidence-based challenges to the continued recommendation and use of peroxidatively-susceptible polyunsaturated fatty acid-rich culinary oils for high-temperature frying practises: Experimental revelations focused on toxic aldehydic lipid oxidation products. *Frontiers in Nutrition*, *8*, 711640. https://doi.org/10.3389/fnut.2021.711640

Gruber, S., Colbach, N., Barbottin, A., & Pekrun, C. (2008). Post-harvest gene escape and approaches for minimizing it. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, *3*, 1–17. https://doi.org/10.1079/PAVSNNR20083015

Hansen, L. B., Siegismund, H. R., & Jorgensen, R. B. (2001). Introgression between oilseed rape (Brassica napus L.) and its weedy relative B. rapa L. in a natural population. *Genetic Resources and Crop Evolution*, *48*(6), 621–627. https://doi.org/10.1023/A:1013825816443

Hansen, L. B., Siegismund, H. R., & Jorgensen, R. B. (2003). Progressive introgression between Brassica napus (oilseed rape) and B. rapa. *Heredity*, *91*(3), 276–283. https://doi.org/10.1038/sj.hdy.6800335

Harmon, D. L., & Swanson, K. C. (2020). Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal*, *14*, S17–S28. https://doi.org/10.1017/S1751731119003136

Harwood, J. L. (2005). *Plant lipids: Biology, utilisation and manipulation* (pp. 27–66). Blackwell Publishing.

Haslam, T. M., & Kunst, L. (2013). Extending the story of very-long-chain fatty acid elongation. *Plant Science*, *210*, 93–107. https://doi.org/10.1016/j.plantsci.2013.05.008

Hecht, M., Oehen, B., Schulze, J., Brodmann, P., & Bagutti, C. (2014). Detection of feral GT73 transgenic oilseed rape (Brassica napus) along railway lines on entry routes to oilseed factories in Switzerland. *Environmental Science and Pollution Research*, *21*(2), 1455–1465. https://doi.org/10.1007/s11356-013-1881-9

Hixson, S. M., Shukla, K., Campbell, L. G., Hallett, R. H., Smith, S. M., Packer, L., & Arts, M. T. (2016). Long-chain omega-3 polyunsaturated fatty acids have developmental effects on the crop pest, the cabbage white butterfly *Pieris rapae*. *PLoS One*, *11*(3), e0152264.

Hong, J., Bledsoe, J. W., Overturf, K. E., Lee, S., Iassonova, D., & Small, B. C. (2022). LatitudeTM oil as a sustainable alternative to dietary fish oil in rainbow trout (Oncorhynchus mykiss): Effects on filet fatty acid profiles, intestinal histology, and plasma biochemistry [original research]. *Frontiers in Sustainable Food Systems*, *6*, 837628. https://doi.org/10.3389/fsufs.2022.837628

Hulter, N., & Wackernagel, W. (2008). Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of Acinetobacter baylyi. *Molecular Microbiology*, *67*(5), 984–995. https://doi.org/10.1111/j.1365-2958.2007.06096.x

Jakobsson, A., Westerberg, R., & Jacobsson, A. (2006). Fatty acid elongases in mammals: their regulation and roles in metabolism. *Progress in Lipid Research*, *45*(3), 237–249. https://doi.org/10.1016/j.plipres.2006.01.004

Jørgensen, R. B. (2007). Oilseed rape: Co-existence and gene flow from wild species. *Advances in Botanical Research: Incorporating Advances in Plant Pathology*, *45*, 451–464. https://doi.org/10.1016/S0065-2296(07)45016-9

Jørgensen, R. B. A. H., Hansen, L. B., Johannessen, M., Andersen, B., & Hauser, T. P. (2004). Gene introgression and consequences in brassica. In *Introgression from genetically modified plants into wild relatives* (pp. 253–262). CAB International. https://doi.org/10.1079/9780851998169.0000

Katsuta, K., Matsuo, K., Yoshimura, Y., & Ohsawa, R. (2015). Long-term monitoring of feral genetically modified herbicide-tolerant Brassica napus populations around unloading Japanese ports. *Breeding Science*, *65*(3), 265–275. https://doi.org/10.1270/jsbbs.65.265

Kenny, F. S., Pinder, S. E., Ellis, I. O., Gee, J. M., Nicholson, R. I., Bryce, R. P., & Robertson, J. F. (2000). Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. *International Journal of Cancer*, *85*(5), 643–648. https://doi.org/10.1002/(SICI)1097-0215(20000301)85:5<643::AID-IJC8>3.0.CO;2-Z

Knispel, A. L., McLachlan, S. M., Van Acker, R. C., & Friesen, L. F. (2008). Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Science*, *56*(1), 72–80. https://doi.org/10.1614/Ws-07-097.1

Laforest, M., Martin, S., Soufiane, B., Bisaillon, K., Maheux, L., Fortin, S., James, T., Miville, D., Marcoux, A., & Simard, M. J. (2022). Distribution and genetic characterization of bird rape mustard (Brassica rapa) populations and analysis of glyphosate resistance introgression. *Pest Management Science*, *78*(12), 5471–5478. https://doi.org/10.1002/ps.7170

Lecoq, E., Holt, K., Janssens, J., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2007). General surveillance: Roles and responsibilities the industry view. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *2*(1), 25–28. https://doi.org/10.1007/s00003-007-0244-1

Lehtovaara, V. J., Valtonen, A., Sorjonen, J., Hiltunen, M., Rutaro, K., Malinga, G. M., Nyeko, P., & Roininen, H. (2017). The fatty acid contents of the edible grasshopper Ruspolia differens can be manipulated using artificial diets. *Journal of Insects as Food and Feed*, *3*(4), 253–262. https://doi.org/10.3920/Jiff2017.0018

Lemke, S. L., Vicini, J. L., Su, H., Goldstein, D. A., Nemeth, M. A., Krul, E. S., & Harris, W. S. (2010). Dietary intake of stearidonic acid-enriched soybean oil increases the omega-3 index: randomized, double-blind clinical study of efficacy and safety. *American Journal of Clinical Nutrition*, *92*, 766–775. https://doi.org/10.3945/ajcn.2009.29072

Leonard, A. E., Pereira, S. L., Sprecher, H., & Huang, Y. S. (2004). Elongation of long-chain fatty acids. *Progress in Lipid Research*, *43*(1), 36–54. https://doi.org/10.1016/s0163-7827(03)00040-7

Li, G., Sinclair, A. J., & Li, D. (2011). Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. *Journal of Agricultural and Food Chemistry*, *59*(5), 1871–1881. https://doi.org/10.1021/jf104154q

Liu, Y. B., Wei, W., Ma, K. P., Li, J. S., Liang, Y. Y., & Darmency, H. (2013). Consequences of gene flow between oilseed rape (Brassica napus) and its relatives. *Plant Science*, *211*, 42–51. https://doi.org/10.1016/j.plantsci.2013.07.002

Londo, J. P., Bautista, N. S., Sagers, C. L., Lee, E. H., & Watrud, L. S. (2010). Glyphosate drift promotes changes in fitness and transgene gene flow in canola (Brassica napus) and hybrids. *Annals of Botany*, *106*(6), 957–965. https://doi.org/10.1093/aob/mcq190

Londo, J. P., Bollman, M. A., Sagers, C. L., Lee, E. H., & Watrud, L. S. (2011). Glyphosate-drift but not herbivory alters the rate of transgene flow from single and stacked trait transgenic canola (Brassica napus) to nontransgenic B. napus and B. rapa. *The New Phytologist*, *191*(3), 840–849. https://doi.org/10.1111/j.1469-8137.2011.03706.x

Luijten, S. H., Schidlo, N. S., Meirmans, P. G., & de Jong, T. J. (2015). Hybridisation and introgression between Brassica napus and B. rapa in The Netherlands. *Plant Biology*, *17*(1), 262–267. https://doi.org/10.1111/plb.12197

Lutman, P. J., Berry, K., Payne, R. W., Simpson, E., Sweet, J. B., Champion, G. T., May, M. J., Wightman, P., Walker, K., & Lainsbury, M. (2005). Persistence of seeds from crops of conventional and herbicide tolerant oilseed rape (Brassica napus). *Proceedings of the Royal Society B: Biological Sciences*, *272*(1575), 1909–1915. https://doi.org/10.1098/rspb.2005.3166

Lutman, P. J. W., Freeman, S. E., & Pekrun, C. (2003). The long-term persistence of seeds of oilseed rape (Brassica napus) in arable fields. *Journal of Agricultural Science*, *141*, 231–240. https://doi.org/10.1017/S0021859603003575

Lutman, P. J. W., Sweet, J., Berry, K., Law, J., Payne, R., Simpson, E., Walker, K., & Wightman, P. (2008). Weed control in conventional and herbicide tolerant winter oilseed rape (Brassica napus) grown in rotations with winter cereals in the UK. *Weed Research*, *48*(5), 408–419. https://doi.org/10.1111/j.1365-3180.2008.00643.x

Meffin, R., Duncan, R. P., & Hulme, P. E. (2015). Landscape-level persistence and distribution of alien feral crops linked to seed transport. *Agriculture Ecosystems & Environment*, *203*, 119–126. https://doi.org/10.1016/j.agee.2015.01.024

Messéan, A., Sausse, C., Gasquez, J., & Darmency, H. (2007). Occurrence of genetically modified oilseed rape seeds in the harvest of subsequent conventional oilseed rape over time. *European Journal of Agronomy*, *27*(1), 115–122. https://doi.org/10.1016/j.eja.2007.02.009

Millar, A. A., & Kunst, L. (1997). Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme. *The Plant Journal*, *12*(1), 121–131. https://doi.org/10.1046/j.1365-313x.1997.12010121.x

Miner-Williams, W. M., Stevens, B. R., & Moughan, P. J. (2014). Are intact peptides absorbed from the healthy gut in the adult human? *Nutrition Research Reviews*, *27*(2), 308–329. https://doi.org/10.1017/S0954422414000225

Mok, C. H., & Urschel, K. L. (2020). Invited review - amino acid requirements in horses. *Asian-Australasian Journal of Animal Sciences*, *33*(5), 679–695. https://doi.org/10.5713/ajas.20.0050

Nishizawa, T., Nakajima, N., Tamaoki, M., Aono, M., Kubo, A., & Saji, H. (2016). Fixed-route monitoring and a comparative study of the occurrence of herbicide-resistant oilseed rape (Brassica napus L.) along a Japanese roadside. *Gm Crops & Food-Biotechnology in Agriculture and the Food Chain*, *7*(1), 20–37. https://doi.org/10.1080/21645698.2016.1138196

Norris, C. S. J., Parker, J., & Law, L. (2004). *Implications for hybridization and introgression between oilseed rape (Brassica napus) and wild turnip (B. rapa) from an agricultural perspective*. CAB International. https://doi.org/10.1079/9780851998169.0107

OECD (Organisation for Economic Co-operation and Development). (2011). Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (Canola): key food and feed nutrients, anti-nutrients and toxicants (ENV/JM/MONO(2011)55). (Series on the Safety of Novel Foods and Feeds, Issue OECD). https://one.oecd.org/document/env/jm/mono(2011)55/en/pdf

Pandolfo, C. E., Presotto, A., Carbonell, F. T., Ureta, S., Poverene, M., & Cantamutto, M. (2016). Transgenic glyphosate-resistant oilseed rape (Brassica napus) as an invasive weed in Argentina: detection, characterization, and control alternatives. *Environmental Science and Pollution Research*, *23*(23), 24081–24091. https://doi.org/10.1007/s11356-016-7670-5

Peltonen-Sainio, P., & Jauhiainen, L. (2014). Lessons from the past in weather variability: sowing to ripening dynamics and yield penalties for northern agriculture from 1970 to 2012. *Regional Environmental Change*, *14*(4), 1505–1516. https://doi.org/10.1007/s10113-014-0594-z

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P. H., & Huet, S. (2008). Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *Journal of Applied Ecology*, *45*(2), 476–485. https://doi.org/10.1111/j.1365-2664.2007.01358.x

Pivard, S., Demsar, D., Lecomte, J., Debeljak, M., & Dzeroski, S. (2008). Characterizing the presence of oilseed rape feral populations on field margins using machine learning. *Ecological Modelling*, *212*(1–2), 147–154. https://doi.org/10.1016/j.ecolmodel.2007.10.012

Saji, H., Nakajima, N., Aono, M., Tamaoki, M., Kubo, A., Wakiyama, S., Hatase, Y., & Nagatsu, M. (2005). Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. *Environmental Biosafety Research*, *4*(4), 217–222. https://doi.org/10.1051/ebr:2006003

Santos-Hernandez, M., Miralles, B., Amigo, L., & Recio, I. (2018). Intestinal signaling of proteins and digestion-derived products relevant to satiety. *Journal of Agricultural and Food Chemistry*, *66*(39), 10123–10131. https://doi.org/10.1021/acs.jafc.8b02355

Schulze, J., Frauenknecht, T., Brodmann, P., & Bagutti, C. (2014). Unexpected diversity of feral genetically modified oilseed rape (Brassica napus L.) despite a cultivation and import ban in Switzerland. *PLoS One*, *9*(12), e114477. https://doi.org/10.1371/journal.pone.0114477

Senger, T., M., L., Kunze, I., Haertel, H. A., Bremmer, S., Breazeale, S., Bauer, J., Vrinten, P., Stymne, S., & Lindberg, Y. J. (2016). Materials and methods for PUFA production, and PUFAcontaining compositions (WO Patent No. W. I. P. O. (WIPO)).

Squire, G. R., Breckling, B., Pfeilstetter, A. D., Jorgensen, R. B., Lecomte, J., Pivard, S., Reuter, H., & Young, M. W. (2011). Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potential as a reservoir of transgene persistence. *Environmental Science and Pollution Research*, *18*(1), 111–115. https://doi.org/10.1007/s11356-010-0376-1

Tang, T., Chen, G., Bu, C., Liu, F., Liu, L., & Zhao, X. (2018). Transgene introgression from Brassica napus to different varieties of Brassica juncea. *Plant Breeding*, *137*(2), 171–180. https://doi.org/10.1111/pbr.12567

Vanbruchem, J., Rouwers, S. M. G., Bangma, G. A., Leffering, C. P., & Vanadrichem, P. W. M. (1985). Digestion of proteins of varying degradability in sheep 0.1. Fermentation in and rate of passage from the reticulorumen. *Netherlands Journal of Agricultural Science*, *33*(3), 263–272.

Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological implications of lipid oxidation products. *Journal of the American Oil Chemists' Society*, *94*(3), 339–351. https://doi.org/10.1007/s11746-017-2958-2

Vo, L. L. G., Galkanda-Arachchige, H. S. C., Iassonova, D. R., & Davis, D. A. (2021). Efficacy of modified canola oil to replace fish oil in practical diets of Pacific white shrimp. *Aquaculture Research*, *52*(6), 2446–2459. https://doi.org/10.1111/are.15094

Warwick, S. I., Légère, A., Simard, M. J., & James, T. (2008). Do escaped transgenes persist in nature?: The case of an herbicide resistance transgene in a weedy Brassica rapa population. *Molecular Ecology*, *17*(5), 1387–1395. https://doi.org/10.1111/j.1365-294X.2007.03567.x

Warwick, S. I., Simard, M. J., Légère, A., Beckie, H. J., Braun, L., Zhu, B., Mason, P., Séguin-Swartz, G., & Stewart, C. N. (2003). Hybridization between transgenic Brassica napus L. and its wild relatives: Brassica rapa L., Raphanus raphanistrum L., Sinapis arvensis L., and Erucastrum gallicum (Willd.) O.E. Schulz. *Theoretical and Applied Genetics*, *107*(3), 528–539. https://doi.org/10.1007/s00122-003-1278-0

Watrud, L. S., King, G., Londo, J. P., Colasanti, R., Smith, B. M., Waschmann, R. S., & Lee, E. H. (2011). Changes in constructed brassica communities treated with glyphosate drift. *Ecological Applications*, *21*(2), 525–538. https://doi.org/10.1890/09-2366.1

Weldon, A., Davis, D. A., Rhodes, M., Morey, A., Iassonova, D., Roy, L. A., & Chen, L. Q. (2023). Use of genetically modified canola oil as a replacement for fish oil in practical diets for Whiteleg shrimp Litopeneaus vannamei reared in green water conditions. *Aquaculture Research*. https://doi.org/10.1155/2023/2999827

Windels, P., Alcalde, E., Lecoq, E., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2009). General surveillance for import and processing: the EuropaBio approach. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *3*(2), 14–16. https://doi.org/10.1007/s00003-009-0359-7

Yilmaz, J. L., Lim, Z. L., Beganovic, M., Breazeale, S., Andre, C., Stymne, S., Vrinten, P., & Senger, T. (2017). Determination of substrate preferences for desaturases and Elongases for production of docosahexaenoic acid from oleic acid in engineered canola. *Lipids*, *52*(3), 207–222. https://doi.org/10.1007/s11745-017-4235-4

Yoshimura, Y., Beckie, H. J., & Matsuo, K. (2006). Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, *5*(2), 67–75. https://doi.org/10.1051/ebr:2006019

Zurier, R. B., Rossetti, R. G., Jacobson, E. W., Demarco, D. M., Liu, N. Y., Temming, J. E., White, B. M., & Laposata, M. (1996). Gamma-linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. *Arthritis & Rheumatism*, *39*(11), 1808–1817. https://doi.org/10.1002/art.1780391106

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Barro, F., Braeuning, A., de Maagd, R., Epstein, M. M., Frenzel, T., Gallois, J.-L., Koning, F., Messéan, A., Moreno, F. J., Nogué, F., Savoini, G., Schulman, A. H., Tebbe, C., Veromann, E., Ardizzone, M., Bonatti, M., De sanctis, G., … Gómez Ruiz, J. A. (2025). Assessment of genetically modified oilseed rape LBFLFK (application EFSA-GMO-DE-2019-157). *EFSA Journal*, *23*(11), e9692. https://doi.org/10.2903/j.efsa.2025.9692

APPENDIX A

Additional studies

Study identification	Title
Study report 30C0342_16S120	EPA + DHA Canola Event LBFLFK RBD Oil Repeated-Dose Toxicity Study in Wistar Rats Administration by Gavage for 4 Weeks
Study report 201703-008R	Germination and fatty acid profile of stored LBFLFK seeds of EPA + DHA canola

APPENDIX B

List of relevant publications identified by the applicant through literature searches (January 2009 – December 2023)

The literature search provided by the applicant on June 2024 was further complemented with an internal literature search on 22 August 2025 to identify any publication not previously identified that might challenge the current conclusions presented in this scientific opinion. No additional relevant publications were identified by the internal search.

Reference

Andre, C., Buesen, R., Riffle, B., Wandelt, C., Sottosanto, J. B., Marxfeld, H., Strauss, V., van Ravenzwaay, B., and Lipscomb, E. A. (2019). Safety assessment of EPA + DHA canola oil by fatty acid profile comparison to various edible oils and fat-containing foods and a 28-day repeated dose toxicity study in rats. *Food and Chemical Toxicology*, *124*, 168–181.

Jeongwhui, H., Bledsoe Jacob, W., Seunghan, L., Small Brian, C., Overturf Kenneth, E., and Diliara, I. (2022). Latitude (TM) Oil as a Sustainable Alternative to Dietary Fish Oil in Rainbow Trout (Oncorhynchus mykiss): Effects on Filet Fatty Acid Profiles, Intestinal Histology, and Plasma Biochemistry. *Frontiers in Sustainable Food Systems*, (42022 MAR 2022) Vol. 6.

Gia Vo, L. L., Harsha, S. C., Arachchige, G., Iassonova, D. R., and Davis, D. R. (2021). Efficacy of modified canola oil to replace fish oil in practical diets of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Research*, *52*, 2446–2459.

Weldon, A., Allen, D. D., Rhodes, M., Morey, A., Iassonova, D., and Roy, L. A. Use of genetically modified canola oil as a replacement for fish oil in practical diets for Whiteleg Shrimp *Litopeneaus vannamei* reared in green water conditions. *Aquaculture Research*, 2023, 2999827. https://doi.org/10.1155/2023/2999827

APPENDIX C

Intended uses (selected foods as proposed by the applicant) based on FoodEx2 Food Classification System[a], and specific maximum concentrations of relevant fatty acids in the foods where RBD LBFLFK oil is to be added

		Maximum level (mg/100 g or 100 mL)[b]		
FoodEx2 name	**FoodEx2 level[a]**	**EPA + DHA + DPA**	**SDA**	**GLA**
Bread and similar products	L2	166	8	54
Fine bakery wares	L2	331	15	108
Raw doughs and pre-mixes	L3	331	15	108
Sandwich and sandwich-like dishes	L4	331	15	108
Pizza and pizza-like dishes	L4	331	15	108
Savoury pies and tarts	L4	331	15	108
Breakfast cereals	L2	132	6	43
Carbohydrate-rich energy products for sports people	L4	265	12	86
Processed cheese and spreads	L3	331	15	108
Savoury sauces	L3	331	15	108
Chocolate and chocolate products	L3	331	15	108
Basic sweet masses	L3	331	15	108
Sweet bars and other formed sweet masses	L3	331	15	108
Dairy imitates	L3	83	4	27
Processed eggs	L2	331	15	108
Egg based dishes	L4	331	15	108
Prepared mixed egg/meat/fish/vegetable salad	L4	331	15	108
Butter[c]	L3	795	37	258
Butter and margarine/oil blends[c]	L4	795	37	258
Shortening and similar baking fats[c]	L4	795	37	258
Margarines and similar[c]	L3	795	37	258
Animal and vegetable fats/oils[c]	L2	795	37	258
Salad dressing[c]	L3	795	37	258
Fish and seafood processed	L2	331	15	108
Fish and seafood-based dishes	L4	331	15	108
Fish soup	L4	331	15	108
Dairy ice creams and similar	L3	331	15	108
Water-based desserts spoonable	L3	66	3	22
Dairy desserts spoonable	L3	66	3	22
Other desserts spoonable	L3	66	3	22
Hard candies	L4	662	31	215
Fruit/vegetable spreads and similar	L3	66	3	22
Canned-tinned meat	L2	331	15	108
Preserved or partly preserved sausages	L3	331	15	108
Meat specialties	L2	331	15	108
Processed whole meat products	L2	331	15	108
Marinated meat	L2	331	15	108
Meat based dishes	L4	331	15	108
Meat soup	L4	331	15	108
Prepared meat salad	L4	331	15	108
Fermented milk or cream	L2	83	4	27
Evaporated milk (liquid, unsweetened)[d]	L4	207	10	67
Condensed milk (sometimes with added sugar)[d]	L4	248	12	81
Milk and dairy powders[d]	L3	745	35	242
Flavoured milks	L4	83	4	27
Cream and cream products	L3	83	4	27

(Continued)

FoodEx2 name	FoodEx2 level[a]	Maximum level (mg/100 g or 100 mL)[b]		
		EPA + DHA + DPA	SDA	GLA
Buttermilk	L3	83	4	27
Whey	L3	83	4	27
Baked milk and similar	L3	83	4	27
Dairy snacks	L3	83	4	27
Single meal replacement for weight reduction	L4	83	4	27
Flavoured bottled water	L4	33	2	11
Fortified bottled water	L4	33	2	11
Water-based beverages	L2	33	2	11
Liquid drinks bases (including concentrates and home-made preparations)[d]	L4	232	11	75
Powdered drink bases[d]	L4	414	19	135
Coffee imitate beverages	L3	33	2	11
Tea infusion with added flavouring ingredients	L4	33	2	11
Carbohydrate-electrolyte solutions for sports people	L4	33	2	11
Candied or sugar preserved nuts	L4	331	15	108
Nut/seeds paste/emulsions/mass	L4	331	15	108
Tree nuts	L3	331	15	108
Pasta and similar products	L3	132	6	43
Pasta-based dishes, uncooked	L4	132	6	43
Pasta-based dishes, cooked	L4	132	6	43
Prepared pasta salad	L4	132	6	43
Meat imitates	L3	331	15	108
Protein and protein components for sports people	L4	331	15	108
Fruit/vegetable juices and nectars	L2	66	3	22
Fruit/vegetable juice concentrate[d]	L3	199	9	65
Fruit/vegetable juice powder[d]	L3	828	38	269
Liquid or gel separated from plant RPCs	L2	66	3	22
Extracts of plant origin	L2	66	3	22
Fried or extruded cereal, seed or root-based products	L2	331	15	108
Soft candies and analogues	L4	265	12	86
Soups (dry mixture uncooked)[d]	L3	199	9	65
Table-top sweeteners formulations	L2	662	31	215
Other sweetening ingredients	L3	662	31	215
Artificial sweeteners (e.g. aspartame, saccharine)	L2	662	31	215
Dessert sauces/toppings	L2	331	15	108
Syrups (molasses and other syrups)	L3	331	15	108
Sucrose (common sugar)	L4	265	12	86

Abbreviation: LBFLFK oil, oil produced from oilseed rape LBFLFK.

[a]The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship (EFSA, 2015).

[b]Maximum levels of EPA, DHA, DPA, SDA and GLA estimated based on LBFLFK oil composition (EPA: 4.081%, DHA: 0.352%, DPA: 2.191%, SDA:0.307%, GLA: 2.153%) as shown in Table 6 of the scientific opinion. Fatty acid concentrations were calculated from the percentage data, assuming that oil is made of 100% FAs. These levels were considered during the dietary intake assessment to conclude on the safety of LBFLFK oil in humans.

[c]Considering the potential safety concerns identified for frying or other high-temperature uses of oils rich in PUFAs (see Section 3.5.2.1 in the scientific opinion), these foods should not be used for high-temperature applications.

[d]For these foods a reconstitution factor was applied before estimating the final concentrations of the fatty acids. Reconstitution factors were obtained from EFSA (2018): https://zenodo.org/record/1256085#.X3eLyThFahd. For 'Milk and dairy powders' the reconstitution factor used was the average of those used for skimmed, semi-skimmed and full-fat milk powder. For "Soups (dry mixture uncooked)" use level was expressed on a dried basis, therefore, no reconstitution factor was required.

ANNEXES

Annex A. Food and Feed safety assessment