SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-NL-2010-87) for the placing on the market of genetically modified herbicide tolerant oilseed rape GT73 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives) under Regulation (EC) No 1829/2003 from Monsanto[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

This scientific opinion is a risk assessment for the placing on the market of the genetically modified (GM) herbicide-tolerant oilseed rape (OSR) GT73 for food containing or consisting of, and food produced from or containing ingredients produced from, OSR-GT73. OSR-GT73 contains a single insert consisting of the *gox*v247 and CP4 *epsps* expression cassettes. Both proteins confer tolerance against glyphosate-based-herbicides. Bioinformatic analyses of inserted DNA and flanking regions did not raise safety concerns. Levels of CP4 EPSPS and GOXv247 proteins in OSR-GT73 were analysed and the stability of the genetic modification was demonstrated. No biologically relevant differences were identified in the compositional/agronomic/phenotypic characteristics of OSR-GT73 compared with its conventional counterpart, except for the newly expressed proteins. No indication of potential concerns over the safety of the newly expressed CP4-EPSPS and GOXv247 proteins or the occurrence of unintended effects were identified in either OSR-GT73 pollen/pollen-containing dietary supplements or the adventitious presence of trace levels of seeds in human foods. An equivalent assessment with isolated seed protein could not be made because of the lack of availability of relevant consumption and safety data. There are no indications of increased establishment and spread of feral OSR-GT73 plants, or of hybridising wild relatives, unless exposed to glyphosate-based-herbicides. Potential interactions of feral plants with the biotic/abiotic environment do not raise concerns. Environmental risks of horizontal gene transfer into bacteria were not identified. The monitoring plan and reporting intervals are in line with the intended uses. The environmental risk assessment of OSR-GT73 did not identify any safety concerns, in the context of its intended uses. While the Panel is not in a position to conclude on the safety of OSR pollen as such, it concludes that the genetic modification in OSR-GT73 does not constitute an additional health risk if OSR-GT73 pollen were to replace non-GM OSR pollen.

[1] On request from the Competent Authority of the Netherlands for an application (EFSA-GMO-NL-2010-87) submitted by Monsanto, Question No EFSA-Q-2010-01088, adopted on 23 January 2013.

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KEY WORDS

GMO, oilseed rape, GT73, herbicide tolerant, human and animal health, food, Regulation (EC) No 1829/2003.

SUMMARY

Following the submission of an application (EFSA-GMO-NL-2010-87) under Regulation (EC) No 1829/2003[4] from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a Scientific Opinion on the safety of genetically modified (GM) herbicide tolerant oilseed rape GT73 (Unique Identifier MON-ØØØ73-7) for food containing or consisting of, and food produced from or containing ingredients produced from, GM oilseed rape GT73 (with the exception of refined oil and food additives).

In delivering its Scientific Opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2010-87, additional information supplied by the applicant, scientific comments submitted by the Member States, and relevant scientific publications. Further information was taken into account from previous applications for placing on the market under the European Union (EU) regulatory procedures of oilseed rape GT73. The scope of application EFSA-GMO-NL-2010-87 is for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives) within the EU, as for any other non-GM oilseed rape, but excluding cultivation in the EU. The EFSA GMO Panel evaluated oilseed rape GT73 with reference to the intended uses and appropriate principles described in its Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed, and on the post-market environmental monitoring of GM plants. The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analysis of composition, phenotypic and agronomic characteristics was undertaken, and the safety of the new proteins and the whole food was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. Evaluation of the environmental impacts and the post-market environmental monitoring plan were undertaken.

Oilseed rape GT73 has been the subject of earlier risk assessment evaluations by the EFSA GMO Panel with the scope: i) import and processing in 2004; and ii) renewal of the authorisation for continued marketing of existing food and food ingredients produced from oilseed rape GT73, and feed materials, feed additives and food additives produced from oilseed rape GT73 in 2009. The EFSA GMO Panel concluded in its Scientific Opinions that GM oilseed rape GT73 is unlikely to have an adverse effect on human and animal health and on the environment, in the context of its proposed uses.

In addition, EFSA published a technical report on a safety analysis of pollen derived from oilseed rape GT73 in food or as food in 2012. In this report, EFSA concluded that considering the data available, no indication of potential concerns over the safety of the newly expressed CP4 EPSPS and GOXv247 proteins, or the occurrence of unintended effects in oilseed rape GT73 pollen have been identified that would raise safety concerns.

The molecular characterisation data established that the oilseed rape GT73 contains one copy of an intact *goxv*247 expression cassette and a CP4 *epsps* cassette in a single locus. No other parts of the plasmid used for transformation are present in oilseed rape GT73. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety issues. The levels of CP4 EPSPS and GOXv247 proteins in oilseed rape GT73 have been sufficiently analysed and the stability of the genetic modification has been demonstrated.

The comparative analysis indicated that no biologically relevant differences were identified in the compositional, agronomic or phenotypic characteristics of oilseed rape GT73 compared with its conventional counterpart, except for the newly expressed CP4 EPSPS and GOXv247 proteins.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1-23.

Updates of the bioinformatic studies confirmed previous findings indicating no similarities of the CP4 EPSPS and GOXv247 proteins to known toxic proteins and allergens.

The EFSA GMO Panel concludes that, considering the data available, no indication of potential concerns over the safety of the newly expressed CP4 EPSPS and GOXv247 proteins or the occurrence of unintended effects have been identified in either oilseed rape GT73 pollen/pollen-containing dietary supplements or the adventitious presence of trace levels of seeds in human foods. An equivalent assessment with isolated seed protein could not be made because of the lack of availability of relevant consumption and safety data.

The safety assessment identified no concerns regarding the potential allergenicity of oilseed rape GT73. In addition, several nutritional studies previously assessed by the EFSA GMO Panel confirmed that oilseed rape GT73 is as nutritious as its conventional counterpart (EFSA, 2004, 2009a).

As this application does not cover cultivation of oilseed rape GT73, there is no requirement for scientific information on possible environmental effects associated with the cultivation of oilseed rape GT73. In the event of the accidental release into the environment of viable oilseed rape GT73 seeds during transport and processing for food uses, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape GT73, unless those plants are exposed to glyphosate-based herbicides. Likewise, evidence indicates that hybridising wild relatives that may theoretically have acquired the herbicide tolerance trait through vertical gene flow are neither more likely to establish, nor more likely to spread than their non-GM comparators in the absence of glyphosate-based herbicides. Considering the intended uses of oilseed rape GT73, potential interactions of feral oilseed rape GT73 plants with the biotic and abiotic environment are not considered to be an issue due to the low levels of exposure. Owing to the intended uses of oilseed rape GT73, the level of exposure of bacteria occurring in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape GT73 is expected to be low. The unlikely but theoretically possible transfer of the recombinant genes from oilseed rape GT73 to bacteria does not raise concerns. Furthermore, tolerance and resistance to glyphosate is widespread among bacteria occurring in the environment making it unlikely that horizontal gene transfer would add to this natural background. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of oilseed rape GT73. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

While the EFSA GMO Panel is not in a position to conclude on the safety of oilseed rape pollen as such, it concludes that the genetic modification in oilseed rape GT73 does not constitute an additional health risk if oilseed rape GT73 pollen were to replace non-GM oilseed rape pollen. The environmental risk assessment of oilseed rape GT73 did not identify any safety concerns, in the context of its intended uses.

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BACKGROUND

On 31 August 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (EFSA-GMO-NL-2010-87) for authorisation of the genetically modified (GM) herbicide tolerant oilseed rape GT73 (Unique Identifier MON-ØØØ73-7) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003. The scope of this application covers food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives) and excludes cultivation.

The EFSA GMO Panel has previously issued Scientific Opinions on oilseed rape GT73 related to: i) a notification C/NL/98/11 for the placing on the market of herbicide tolerant oilseed rape GT73 for import and processing, under part C of Directive 2001/18/EC (EFSA, 2004); and ii) the renewal of the authorisation for continued marketing of existing (a) food and food ingredients produced from oilseed rape GT73, and of (b) feed materials, feed additives and food additives produced from oilseed rape GT73, under Regulation (EC) No 1829/2003 (EFSA, 2009a). In these Scientific Opinions, the EFSA GMO Panel concluded that oilseed rape GT73 is unlikely to have an adverse effect on human or animal health or on the environment, in the context of its proposed uses. In addition, EFSA published a technical report on a safety analysis of pollen derived from oilseed rape GT73 in food or as food (EFSA, 2012a). In this report, EFSA concluded that considering the data available, no indication of potential concerns over the safety of the newly expressed CP4 EPSPS and GOXv247 proteins, nor the occurrence of unintended effects in oilseed rape GT73 pollen have been identified that could raise safety concerns (EFSA, 2012a).

After receiving the application EFSA-GMO-NL-2010-87 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed both Member States and the European Commission, and made the summary of the application publicly available on the EFSA website[5]. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Article 5(3) of Regulation (EC) No 1829/2003. On 6 July 2011 and 31 October 2011, EFSA received additional information requested under completeness check (requested on 17 November 2010 and 2 August 2011, respectively). On 22 November 2011, EFSA declared the application as valid in accordance with Article 6(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission (EC) and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[6], following the requirements of Article 6(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 22 February 2012) within which to make their opinion known.

The EFSA Scientific Panel on Genetically Modified Organisms (EFSA GMO Panel) carried out a scientific risk assessment of oilseed rape GT73 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives), in accordance with Article 6(6) of Regulation (EC) No 1829/2003. When carrying out the safety evaluation, the EFSA GMO Panel took into account the appropriate principles described in its guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011). Furthermore, the scientific comments of Member States, the additional information provided by the applicant, relevant scientific publications and information from previous applications on oilseed rape GT73 were taken into consideration.

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-01088

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1–38.

On 27 January 2012, 13 June 2012 and 5 July 2012, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 23 April 2012 and 14 September 2012.

In giving its opinion on application EFSA-GMO-NL-2010-87 to the European Commission, the Member States and the applicant, and in accordance with Article 6(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1) and 6(2) of Regulation (EC) No 1829/2003. According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Article 6(6) of that Regulation and thus will be part of the EFSA overall opinion in accordance with Article 6(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of oilseed rape GT73 with the scope for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives) in accordance with Article 6 of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of food containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Article 6(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. INTRODUCTION

The genetically modified (GM) oilseed rape GT73 (Unique Identifier MON-ØØØ73-7) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed (EFSA, 2006a) and on the post-market environmental monitoring (PMEM) of GM plants (EFSA, 2006b, 2011).

Oilseed rape GT73 was developed to express the CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and glyphosate oxidoreductase variant 247 (GOXv247) proteins, both conferring tolerance to glyphosate-based herbicides.

The scope of application EFSA-GMO-NL-2010-87 is for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (including pollen of oilseed rape GT73 and the accidental unintentional presence of viable seeds but excluding refined oil and food additives) and does not include cultivation in the European Union (EU).

The EFSA GMO Panel notes that only seeds and pollen of oilseed rape are currently used in the human food and animal feed chain. Seeds are processed into food-grade vegetable oil, which can therefore be a component of the human diet. The use of refined oil and food additives derived from oilseed rape GT73 has already been notified within the EU[7] and assessed by EFSA (EFSA, 2004, 2009a). The main by-product from oil processing, the mechanically and/or solvent-extracted meal, is used as feed for all classes of livestock, while feed uses of oilseed rape GT73 were evaluated by the EFSA GMO Panel in its previous Scientific Opinions (EFSA, 2004, 2009a). The EFSA GMO Panel is unaware of evidence of the deliberate consumption of whole seeds of oilseed rape. However, occasional adventitious presence of human foods with trace levels of oilseed rape can occur, *e.g.* in mustards. In addition, in 2012, an application for the commercial use of seed protein isolates from oilseed rape, rich in either the cruciferin or napin proteins was made.[8]

The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information obtained from the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information from previous applications for the placing on the EU market of oilseed rape GT73 as well as an EFSA technical report on a safety analysis of pollen derived from oilseed rape GT73 in food or as food, were taken into account (EFSA, 2004, 2009a, 2012a).

2. ISSUES RAISED BY MEMBER STATES

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[9] and have been considered throughout this scientific opinion.

3. MOLECULAR CHARACTERISATION

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs

Oilseed rape GT73 was developed by the *Agrobacterium tumefaciens*-mediated transformation of five- to six-week-old leaves and buds of Westar oilseed rape with plasmid vector PV-BNGT04. The regeneration of the transformed tissue was achieved after a callus phase.[10] The plasmid vector PV-

[7] http://ec.europa.eu/food/dyna/gm_register/index_en.cfm
[8] http://ec.europa.eu/food/food/biotechnology/novelfood/app_list_en.pdf
[9] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2010-01088
[10] Technical Dossier / Section C.1.

BNGT04 included one transfer DNA (T-DNA) which contained two expression cassettes between the right and left borders.[11]

The *gox*v247 expression cassette consisted of the 35S promoter from *Figwort mosaic virus* (FMV); the CTP1 chloroplast transit peptide-encoding sequence derived from the *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase (RuBisCO) small subunit 1A gene; the *gox*v247 synthetic coding sequence encoding the glyphosate oxidoreductase variant GOXv247; and the 3′ untranslated region of the pea RuBisCO small subunit E9 gene, which serves as a transcription terminator. The GOXv247 protein expressed in oilseed rape GT73 was developed from the GOX protein of *Ochrobactrum anthropi* strain LBAA and differs from the wild-type variant in only three amino acid positions out of 431.

The CP4 *epsps* expression cassette consisted of the same FMV promoter used in the *gox*v247 expression cassette; the CTP2 chloroplast transit peptide-encoding sequence of the *epsps* gene from *A. thaliana*; the CP4 *epsps* coding sequence from *A. tumefaciens* strain CP4; and the 3′ untranslated region of the pea RuBisCO small subunit E9 gene, which serves as a transcription terminator.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria: *ori*V, the origin of replication from the broad host range plasmid RK2 for the maintenance of plasmid vector in *Agrobacterium*; *rop*, repressor of primer protein, playing a role in the maintenance of plasmid copy number in *Escherichia coli*; *ori*-pBR322, the origin of replication from plasmid pBR322 required for the maintenance of PV-BNGT04 in *E. coli*; and bacterial promoter, coding and 3′ untranslated sequences of *aad*A from tansposon Tn*7*, an aminoglycoside-modifying enzyme conferring resistance to spectinomycin and streptomycin for selection of the plasmid in *E. coli* and *Agrobacterium*.

3.1.2. Transgene constructs in the genetically modified plant[12]

To determine the structure and copy number of insert(s) in oilseed rape GT73 genomic DNA, a combination of Southern blot analysis and polymerase chain reaction (PCR) was used. Southern analyses were performed using appropriate combinations of restriction endonucleases and seven probes that cover the whole plasmid. The probes corresponding to the different elements of the T-DNA showed the expected hybridisation signals for a single insert with one copy of each expression cassette, whereas no signal was observed with the two probes covering the entire vector backbone.

The nucleotide sequences of the insert as well as both 5′ and 3′ flanking regions were determined. Comparison of the pre-insertion locus in oilseed rape variety Westar with GT73 indicated that the pre-insertion locus was preserved except for the deletion of 40 bp and the addition of 22 bp at the 5′ insert-to-genomic DNA junction.

Bioinformatic analyses of the genomic sequences flanking the insert and the pre-insertion site were carried out to assess any potential interruption of known oilseed rape genes. BLASTn searches were performed against plant EST (Expressed Sequence Tag) database and non-redundant nucleotide database and BLASTx searches against non-redundant amino acid database. The results did not indicate the interruption of any known endogenous gene in oilseed rape GT73. The results also confirmed that the insert is located in the nuclear genome.

The applicant provided a BLASTx analysis of the entire T-DNA insert and its junctions. Using the FARPP database no alignment was found that met or exceeded the Codex Alimentarius (2003) and EFSA (2010) threshold for potential allergenicity. Similarly, bioinformatic analysis revealed no relevant similarities to known toxic proteins. Even in the unlikely event that any of the new open

[11] Technical Dossier / Sections C.2. and C.3.
[12] Technical Dossier / Section D.2.

reading frames (ORFs) at the junctions or within the insert were translated, these analyses did not indicate a safety issue.

3.1.3. Information on the expression of the insert[13]

The levels of CP4 EPSPS and GOXv247 proteins were determined by enzyme-linked immunosorbent assay (ELISA) using leaf and seed samples from field trials across major oilseed rape-growing regions in Canada (1992 and 1993) and in Europe (1994, 1995, 1995/1996). Three of these field trials (1993, 1994, 1995) included both herbicide-treated and untreated plants indicated that glyphosate treatment had no significant effect on the levels of the newly expressed proteins in the seeds. Considering the scope of the application, the CP4 EPSPS and GOXv247 protein levels in seeds are considered the most relevant. The mean CP4 EPSPS level for seeds across all Canadian sites was 34 µg/g fresh weight (fw) (range 13 – 51 µg/g fw); the respective value for the European trials was 22 µg/g fw (range 12 – 37 µg/g fw). The mean GOXv247 level for seed across all Canadian sites was 170 µg/g fresh weight (fw) (range 49 – 379 µg/g fw); the respective value for the European trials was 165 µg/g fw (range 59 – 313 µg/g fw).

3.1.4. Inheritance and stability of inserted DNA[14]

Genetic stability of the inserted DNA was studied over three generations (R_3 and R_5) of oilseed rape GT73 by Southern blot analysis. The restriction enzyme/probe combinations used were sufficient to conclude that the generations tested retained the single copy insert. Analysis of the expression of CP4 EPSPS and GOXv247 proteins over multiple generations indicated phenotypic stability of oilseed rape GT73. The inheritance pattern of the glyphosate tolerance trait was consistent with a single genetic locus segregating in a Mendelian fashion.

3.2. Conclusion

The molecular characterisation data establish that oilseed rape GT73 contains one insert with one copy of each expression cassette. No vector backbone sequences are present in the transformed plant. Bioinformatic analysis of the 5′ and 3′ flanking regions did not reveal disruption of known oilseed rape genes or the creation of ORFs that would raise a safety issue. The potential impacts of the CP4 EPSPS and GOXv247 protein levels, quantified in field trials carried out in Canada and in Europe, are assessed in the food/feed and environment sections. The stability of the inserted DNA was confirmed over three generations. The EFSA GMO Panel considers that all of the molecular data sets are sufficient for the molecular characterisation.

4. COMPARATIVE ANALYSIS

4.1. Evaluation of relevant scientific data

4.1.1. Compositional analysis, agronomic traits and GM phenotype[15]

The information in application EFSA-GMO-NL-2010-87 regarding the comparative analysis of agronomic, phenotypic and compositional data had been provided to EFSA earlier as scientific documentation in the frame of previous applications for the GM herbicide tolerant oilseed rape GT73 with different scopes (EFSA, 2004, 2009a). The original information contained agronomic and phenotypic data obtained from field trials performed with oilseed rape GT73 and the conventional counterpart (Westar) in Canada and Europe over several seasons (from 1992 to 2000), as well as compositional data on the harvested seed material. Glucosinolate level differences, initially observed

[13] Technical Dossier / Section D.3.
[14] Technical Dossier / Section D.5.
[15] Technical dossier / Section D.7.1.

in the 1992-1996 field trials (as reported in EFSA, 2004), were attributed to natural variation, as demonstrated in additional field trials.[16]

4.2. Conclusion

Since the EFSA GMO Panel delivered its earlier opinions on oilseed rape GT73 no new information has appeared on the composition or on the agronomic and phenotypic characteristics of oilseed rape GT73 that would lead the EFSA GMO Panel to change its previous conclusions. Therefore, the EFSA GMO Panel concludes that no biologically relevant differences were identified in the compositional, agronomic and phenotypic characteristics of oilseed rape GT73 compared with its conventional counterpart, except for the newly expressed CP4 EPSPS and GOXv247 proteins.

5. FOOD/FEED SAFETY ASSESSMENT

5.1. Evaluation of relevant scientific data

5.1.1. Toxicological assessment[17]

The data evaluated by the EFSA GMO Panel in the frame of the toxicological assessment of previous applications included data on the safety of the newly expressed proteins, as well as that of the whole GM food/feed. Data previously evaluated by the EFSA GMO Panel pertained to: the structural and functional identity of the CP4 EPSPS and GOXv247 proteins produced in recombinant *E. coli* with those expressed in oilseed rape GT73; bioinformatics analysis comparing the sequences of these proteins and known toxic proteins; the resistance of these proteins to enzymatic degradation by pepsin and pancreatin; and acute oral toxicity studies with these proteins in mice. The EFSA GMO Panel previously concluded that oilseed rape GT73 is unlikely to have an adverse effect on human and animal health, in the context of the proposed uses (EFSA, 2004, 2009a).

In this application EFSA-GMO-NL-2010-87, updated bioinformatics studies were provided. Analyses of the amino acid sequences of the newly expressed proteins CP4 EPSPS and GOXv247 revealed no similarity to known toxic proteins and thus confirmed the results of the previous studies.

The EFSA GMO Panel previously evaluated the safety of the CP4 EPSPS protein in the context of other applications for the placing on the market of GM crops expressing CP4 EPSPS, and no safety concerns were identified (e.g., EFSA-GMO-UK-2004-08, EFSA-GMO-NL-2005-22, EFSA-GMO-CZ-2005-27, EFSA-NL-2006-36). In the case of GOXv247 protein, no such additional information can be gathered from EFSA GMO Scientific Opinions other than the one on oilseed rape GT73.

In the frame of application EFSA-GMO-NL-2010-87, additional information[18] to support the safety assessment of the GOXv247 protein was provided by the applicant in response to a request from the EFSA GMO Panel for a 28-day oral toxicity study in rodents with the GOXv247 protein. This information[16] contained data on: the safety of the donor organism; enzyme specificity; digestibility; level of expression in seed; acute toxicity study; history of use of oilseed rape GT73 since its global commercialisation; and animal studies with the whole food/feed. In addition, the applicant provided a dietary risk assessment based on estimated intakes (calculated with the highest detected values in seed) of the newly expressed GOXv247 (and CP4 EPSPS) protein by consumers through consumption of pollen present in honey and pollen-containing dietary supplements.[19]

[16] Technical dossier / Appendix XII and Appendix XIII
[17] Technical dossier / Section D.7.8. and additional information received in April 2012.
[18] Additional information received in April 2012.
[19] GOXv247 protein estimated intakes amounts to 3.7 and 106 µg GOXv247/kg bodyweight/day in adults from honey and pollen-containing dietary supplements, respectively, and 206 µg GOXv247/kg bodyweight/day in children from dietary pollen supplements. CP4 EPSPS protein estimated intakes amounts to 0.5 and 15.4 µg CP4 EPSPS/kg bodyweight/day in adults from honey and pollen-containing dietary supplements, respectively, and 30 µg CP4 EPSPS/kg bodyweight/day in children from dietary pollen supplements.

The EFSA GMO Panel considers that there are no safety concerns for the presence of GOXv247 in pollen present in honey or pollen-containing dietary supplements taking into account: i) the similarity of GOXv247 to commonly occurring enzymes involved in amino acid biosynthesis as well as lack of similarity with toxins and allergens; ii) its degradation by proteolytic enzymes; iii) lack of indications of toxicity from tests with the whole GM food/feed; and iv) its anticipated levels of intake by consumers. A similar argument applies to the adventitious presence of trace levels of oilseed rape GT73 seeds in human foods.

The EFSA GMO Panel notes that, based on analytical data provided by the applicant, the concentration of GOXv247 in protein extracts of whole seeds could reach levels of 0.5 mg/g.[20] Given the interest in the use of isolated rapeseed protein,[21] a substantially high intake of GOXv247 is foreseen as being possible by the EFSA GMO Panel. In the absence of consumption data and repeated dose toxicity studies with the GOXv247 protein, the EFSA GMO Panel is unable to complete the risk assessment for products of this nature.

There is no new scientific information that would invalidate the previous EFSA GMO Panel conclusions on the toxicological assessment of oilseed rape GT73 (EFSA, 2004, 2009a).

5.1.2. Allergenicity assessment[22]

The EFSA GMO Panel has previously evaluated the structural and functional identity of the CP4 EPSPS and GOXv247 proteins produced in recombinant *E. coli* to those expressed in GT73 oilseed rape, the bioinformatics analysis comparing the sequences of these proteins and known allergic proteins and the resistance of these proteins to enzymatic degradation by pepsin and pancreatin (EFSA, 2004, 2009a).

In this application, updated bioinformatics studies were provided. Analyses of the amino acid sequences of the newly expressed proteins CP4 EPSPS and GOXv247 revealed no relevant similarities to known allergens and thus confirmed the results of the previous studies.

In the present case and based on all the available information, the EFSA GMO Panel identified no safety concerns regarding the potential allergenicity of oilseed rape GT73. In addition, there is no new scientific information that would invalidate the previous EFSA GMO Panel conclusions on the allergenicity assessment of oilseed rape GT73 (EFSA, 2004, 2009a).

5.1.3. Nutritional assessment[23]

The EFSA GMO Panel previously evaluated animal feeding studies in the frame of former applications. The feeding studies were performed in rat, rainbow trout, quail, chicken and lamb (EFSA, 2004, 2009a). These feeding studies support the results of the comparative compositional analysis and confirm that oilseed rape GT73 is as nutritious as its conventional counterpart (EFSA, 2004, 2009a). In the absence of new scientific evidence specific to oilseed rape GT73, the previous EFSA GMO Panel conclusions on oilseed rape GT73 remain valid and applicable.

5.2. Conclusion

Updates of the bioinformatic studies confirmed the previous findings indicating that there are no similarities between the CP4 EPSPS and GOXv247 proteins and known toxic proteins and allergens.

Considering the data available, the EFSA GMO Panel concludes that no indication of potential concerns over the safety of the newly expressed CP4 EPSPS and GOXv247 proteins or the occurrence of unintended effects have been identified in either oilseed rape GT73 pollen/pollen-containing dietary

[20] Technical dossier / Section D.3.
[21] http://ec.europa.eu/food/food/biotechnology/novelfood/app_list_en.pdf
[22] Technical dossier / Section D.7.9.
[23] Technical dossier / Section D.7.10.

supplements or the adventitious presence of trace levels of seeds in human foods. An equivalent assessment with isolated seed protein could not be made because of the lack of availability of relevant consumption and safety data.

The safety assessment identified no concerns regarding the potential allergenicity of oilseed rape GT73. Furthermore, several nutritional studies previously assessed by the EFSA GMO Panel confirmed that oilseed rape GT73 is as nutritious as its conventional counterpart (EFSA, 2004, 2009a).

A review of peer-reviewed scientific data[24] on oilseed rape GT73 and derived food and feed, relevant to the safety assessment, revealed that there was no new information that would require changes to previous EFSA GMO Panel Scientific Opinions on oilseed rape GT73.

The EFSA GMO Panel's conclusions on oilseed rape GT73 in its previous Scientific Opinions remain valid and applicable (EFSA, 2004, 2009a).

6. Environmental risk assessment and monitoring plan

6.1. Evaluation of relevant scientific data

Considering the intended uses of oilseed rape GT73, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds of oilseed rape GT73 during transport and processing for food uses, and with the horizontal gene transfer to bacteria occurring in the environment or human digestive tract. As the scope of the present application excludes cultivation, environmental concerns in the EU pertaining to the use of glyphosate-based herbicides on oilseed rape GT73 do not apply.

6.1.1. Environmental risk assessment

6.1.1.1. Effects on plant fitness due to the genetic modification[25]

In its 2004 Scientific Opinion, the EFSA GMO Panel concluded based on a comparative analysis of agronomic traits and composition of oilseed rape GT73 that "*there was no indication of unintended effects of the genetic modification*", and that "*with the exception for the introduced proteins, there are no differences between GT73 oilseed rape and its appropriate comparators*". The EFSA GMO Panel also indicated that "*studies with GT73 oilseed rape have not shown any enhanced weediness or fitness, except when glyphosate herbicide is applied*" (EFSA, 2004).

Demographic studies of feral oilseed rape have shown the ability of oilseed rape to establish self-perpetuating populations outside agricultural areas, mainly in semi-natural and ruderal habitats in different countries (reviewed by Devos et al., 2012). Oilseed rape is generally regarded as an opportunistic species and can take advantage of disturbed sites due to its potential to germinate and capture resources rapidly. Once established in competition-free germination sites, feral populations become extinct over a period of years. A 10-year survey (1993-2002), along road verges of a motorway revealed that most quadrats showed transient populations lasting one to four years (Crawley and Brown, 2004). These data and data from other demographic studies indicate a substantial turnover of populations of feral oilseed rape: only a small percentage of populations occurs at the same location over successive years, whereas the majority disappears rapidly (Crawley and Brown, 1995, 2004; Charters et al., 1999; Peltzer et al., 2008; Elling et al., 2009; Knispel and McLachlan, 2009; Nishizawa et al., 2009; Squire et al., 2011). If habitats are disturbed on a regular basis by anthropogenic activities such as mowing, herbicide application or soil disturbance, or natural occurrences such as flooding, then feral populations can persist for longer periods (Claessen et al., 2005a; Garnier et al., 2006). The persistence or recurrence of a population in one location is variously attributed to replenishment with fresh seed spills, recruitment from seed emerging from the soil seedbank or shed by resident feral

[24] Additional information received in April 2012.
[25] Technical dossier / Sections B.3. and D.4. and additional information received in September 2012.

adult plants, or redistribution of feral seed from one location to another. While many feral populations observed over multiple years were transient at a local scale (e.g., Crawley and Brown, 1995, 2004; Knispel et al., 2008), this apparent transience is probably counterbalanced on a landscape scale by repeated seed addition and redistribution from various sources (Pivard et al., 2008a,b). On a larger scale in the landscape, feral oilseed rape can thus be considered long lived with a proportion of the populations founded by repeated fresh seed spills from both agricultural fields and transport, and the remainder resulting from the continuous recruitment of seed from local feral soil seedbanks (Pivard et al., 2008a,b).

The above-mentioned demographic studies and surveys monitoring transgene presence in feral oilseed rape populations indicate that feral oilseed rape is generally confined to ruderal habitats and that GM herbicide tolerant (GMHT) oilseed rape also behaves as a typical non-persistent ruderal plant. The ability of oilseed rape to successfully invade natural habitats is limited principally by the availability of seed germination sites and interspecific plant competition (Crawley et al., 1993, 2001; Crawley and Brown, 1995; Hails et al., 2006; Damgaard and Kjaer, 2009). Moreover, in controlled sowings into road verges, field margins and wasteland, very few seedlings survived to maturity due to grazing (e.g., by molluscs) and abiotic stress (Charters et al., 1999). Field studies (such as transplant or seed sowing experiments) have confirmed that herbicide tolerance traits in oilseed rape do not confer a fitness advantage, unless the specific herbicides for which tolerance has been obtained are applied (Crawley et al., 1993, 2001). Crawley et al. (1993, 2001) have assessed the invasive potential of GM plants directly by releasing them into natural habitats and by monitoring their fitness in subsequent generation(s). GMHT oilseed rape introduced into twelve different habitats at three sites across the UK failed to persist in established vegetation: in none of the natural plant communities considered was oilseed rape found after three years even when vegetation had been removed in the first year of sowing (Crawley et al., 1993, 2001). These experiments demonstrated that the genetic modification *per se* does not enhance ecological fitness. Ecophysiological experiments on comparative fitness between the GM plant and its non-GM counterpart and modelling did not indicate that genes conferring herbicide tolerance significantly alter the competitive ability of GM plants (Fredshavn et al., 1995; Warwick et al., 1999, 2004, 2009; Norris and Sweet, 2002; Claessen et al., 2005a,b; Garnier and Lecomte, 2006; Garnier et al., 2006; Simard et al., 2005; Londo et al., 2010). Beckie et al. (2004) showed that GMHT oilseed rape with single or multiple herbicide tolerance traits is not more persistent (weedier) than non-GMHT plants. In addition, greenhouse studies, in which the fitness of oilseed rape volunteers with no, single, or multiple herbicide tolerance was assessed, have shown no or little difference in fitness among oilseed rape plants in the absence of herbicide pressure (Simard et al., 2005). There is also no evidence that tolerance to glyphosate or glufosinate-ammonium enhances seed dormancy, and thus the persistence of GMHT oilseed rape plants, compared with their non-GM comparators (Hails et al., 1997; Sweet et al., 2004; Lutman et al., 2005, 2008; Messéan et al., 2007). Seed dormancy is more likely to be affected by the genetic background of parental genotypes than the acquisition of herbicide tolerance traits (López-Granados and Lutman, 1998; Lutman et al., 2003; Gulden et al., 2004a,b; Gruber et al., 2004; Messéan et al., 2007; Baker and Preston, 2008). The evidence described above indicates that GMHT oilseed rape is neither more likely to survive nor more likely to be more persistent or invasive than its non-GM comparator in the absence of glyphosate or glufosinate-ammonium-based herbicides.

Oilseed rape has hybridising wild relatives (see section 6.1.1.2), but there is no evidence to suggest that herbicide tolerance traits in wild relatives change the plant's behaviour (Norris et al., 2004; Warwick et al., 2008), or the scale and nature of their interactions with associated flora and fauna (Wilkinson and Ford, 2007). The progeny of hybrids of oilseed rape and wild relatives bearing the herbicide tolerance trait does not show any enhanced fitness, persistence or invasiveness, and behaves as their non-GM comparators, unless the herbicides for which tolerance has been obtained are applied (Londo et al., 2010; Watrud et al., 2011).

The EFSA GMO Panel reviewed all relevant scientific literature that has been published since the adoption of its Scientific Opinion in 2004 and concludes that no new information that would require alteration of its previous conclusion on oilseed rape GT73 has become available (see EFSA, 2004, 2009c, 2012a). Therefore, the conclusion that oilseed rape GT73 has no altered agronomic or phenotypic characteristics, except for the herbicide tolerance, is reiterated. Glyphosate tolerant oilseed rape is neither more likely to survive, nor more persistent or invasive, than its non-GM comparators in the absence of glyphosate-based herbicides. The ability of oilseed rape to successfully invade and subsequently persist in ruderal habitats appears to be limited principally by the availability of seed germination sites and interspecific plant competition, and there is no evidence that genes conferring herbicide tolerance significantly alter its competitive ability, except in the presence of the herbicidal active ingredient. The likelihood of unintended environmental effects arising from the establishment, survival and spread of oilseed rape GT73 will therefore not be different from that of non-GM oilseed rape varieties, unless those plants are exposed to glyphosate-based herbicides.

6.1.1.2. Potential for gene transfer[26]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via the dispersal of pollen and seed.

(a) Plant-to-bacteria gene transfer

The EFSA GMO Panel previously evaluated the plant-to-bacteria gene transfer from oilseed rape GT73 to bacteria and the potential environmental consequences of such gene transfer (EFSA, 2004, 2009a). It concluded that: "*in the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health or the environment are expected, as no principally new traits would be introduced into or expressed by natural microbial communities*".

The EFSA GMO Panel reiterates its previous conclusions, as it did not identify properties of the DNA inserted in oilseed rape GT73 that would change the likelihood of horizontal transfer compared with other plant genes. Current scientific knowledge (see EFSA, 2009b for further details) suggests that gene transfer from GM plants to bacteria under natural conditions is extremely unlikely, and that its establishment in recipient genomes would occur primarily through homologous recombination. The CP4 *epsps* and *gox* genes, as expressed in oilseed rape GT73, are of bacterial origin (from *Agrobacterium* sp. strain CP4) and *O. anthropi* strain LBAA, respectively). As natural variants of such genes are already present in bacteria occurring in the environment, homologous recombination and acquisition of the recombinant genes by bacteria will not confer novel properties possibly providing selective advantages to members of the natural microbial communities (Cao et al., 2012; Fan et al., 2012; Sviridov et al., 2012). In environments frequently exposed to glyphosate, bacteria with resistance against this compound may be selected. However, glyphosate tolerance and resistance has been described for several bacterial species and is expected to be common in bacterial communities in the environment. Considering the scope of this application, it should be noted that glyphosate as a herbicidal compound and selective agent for some bacteria is not expected to be present in the main receiving environment, i.e., the gastrointestinal tract of humans. Taking into account the bacterial origin of the CP4 *epsps* and *gox* genes and the activities of their encoded proteins, the limited exposure indicated by the scope of this application, and a highly unlikely but theoretically possible horizontal transfer of these recombinant genes in the background of natural variants of these genes and natural gene transfer processes between bacteria occurring in the environment, potentially adverse effects on human health or the environment were not identified.

Considering the intended uses as food and the above assessment, and in agreement with its previous Scientific Opinions on oilseed rape GT73, the EFSA GMO Panel has not identified any concern associated with horizontal gene transfer from oilseed rape GT73 to bacteria.

[26] Technical dossier / Sections B.2., B.4., D.4. and D.6., and additional information received in September 2012.

(b) Plant-to plant-gene transfer

The EFSA GMO Panel previously evaluated the plant-to-plant gene transfer from feral oilseed rape GT73 plants to cross-compatible plant species and the potential environmental consequences of such gene transfer (EFSA, 2004, 2009c). The EFSA GMO Panel indicated that "*the likelihood for unintended environmental effects due to the establishment and spread of GT73 oilseed rape will not be different from that of traditionally bred oilseed rape. Even if feral populations of GT73 oilseed rape were established and transgene flow occurs at a low frequency to cultivated oilseed rape and/or other Brassicae, a selective advantage only occurs if the complementary herbicide is applied*".

Newly published data since the adoption of the 2004 EFSA GMO Panel Scientific Opinion confirm that seed dispersal is likely to occur and to result in feral GMHT oilseed rape plants in regions where GMHT oilseed rape is cultivated and/or transported (reviewed by Devos et al., 2012). In regions where GMHT oilseed rape is widely grown such as western Canada and the USA, monitoring surveys revealed the widespread occurrence of feral GMHT oilseed rape plants along field margins of agricultural fields, as well as along transportation routes (such as road verges and railway lines). In the study by Yoshimura et al. (2006), approximately two-thirds of the feral plants sampled were transgenic, whereas all feral plants sampled by Knispel et al. (2008) exhibited the presence of the glyphosate or glufosinate-ammonium tolerance traits (or both). In North Dakota (USA), 80% (231/288) of the sampled feral oilseed rape plants expressed at least one herbicide trait (CP4 EPSPS) and phosphinothricin-*N*-acetyltransferase (PAT)): 41% (117/288) of the plants were positive for only CP4 EPSPS and 39% (112/288) were positive for PAT; and 0.7% (2/288) expressed both herbicide tolerance traits (Schafer et al., 2011). The presence of feral GMHT oilseed rape plants was also detected at the port of Vancouver on the west coast of Canada, where most GMHT oilseed rape seed for export is transported by rail (Yoshimura et al., 2006). These data indicate that feral GMHT oilseed rape will be present along roadsides and other ruderal habitats in areas where GMHT oilseed rape is commercially grown and transported as viable seed. Surveys in Japan, where GMHT oilseed rape is currently not grown commercially, performed in and around major ports and along roads leading from these ports to inland processing facilities, reported feral oilseed rape plants with glyphosate or glufosinate-ammonium tolerance, and to a lesser extent both traits (Saji et al., 2005; Aono et al., 2006; Kawata et al., 2009; Nishizawa et al., 2009). The proportion of feral plants that was transgenic varied substantially across years and sampling sites, ranging from 0.2% to 100% (Kawata et al., 2009; Nishizawa et al., 2009). Aono et al. (2006) also reported the presence of *barnase* and *barstar* genes in the progeny of some of the sampled oilseed rape plants. As no GM oilseed rape has been grown for marketing purposes in Japan (Nishizawa et al., 2010), transgene presence could be attributed to the accidental loss and spillage of imported viable GMHT oilseed rape seeds. These data indicate that seed dispersal of GMHT oilseed rape will occur wherever it is transported or cultivated, so that feral plants are likely to be present along transportation routes in all countries cultivating and/or receiving imports of viable seeds of GMHT oilseed rape and in ruderal habitats in areas where GMHT oilseed rape is commercially grown.

Oilseed rape is an outcrossing species with the potential to cross-pollinate other oilseed rape types at varying levels of frequency depending on flowering synchrony, spatial arrangement of plants, presence of pollinator insects and other factors as reviewed by Eastham and Sweet (2004). Feral oilseed rape GT73 plants arising from spilled seeds could therefore pollinate crop plants of non-GM oilseed rape if feral populations are immediately adjacent to field crops (Garnier and Lecomte, 2006). Shed seed from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops. Squire et al. (2011) and Devos et al. (2012) considered that the frequency of such events was likely to be extremely low and concluded that this route of gene flow would not introduce significant numbers of GM plants into farmland or result in any environmental consequences.

Oilseed rape is known to spontaneously hybridise with certain of its sexually compatible wild relatives (Scheffler and Dale, 1994, Eastham and Sweet, 2004, Chèvre et al., 2004; Devos et al., 2009). Several oilseed rape $\times$ wild relative hybrids have been reported in the scientific literature, but under field conditions transgene introgression has only been confirmed only for the progeny of oilseed rape × *Brassica rapa* hybrids (Hansen et al., 2001, 2003; Warwick et al., 2003, 2008; Norris et al., 2004; Jørgensen, 2007). Due to ecological and genetic barriers, not all relatives of oilseed rape share the same potential for hybridisation and transgene introgression (Jenczewski et al., 2003; Chèvre et al., 2004; FitzJohn et al., 2007; Wilkinson and Ford, 2007; Devos et al., 2009; Jørgensen et al., 2009). As no or only very low numbers of viable and fertile hybrids are obtained between oilseed rape and most of its wild relatives under ideal experimental conditions (e.g., through the use of artificial pollination and embryo rescue techniques in laboratory conditions (see FitzJohn et al., 2007)), Wilkinson et al. (2003) concluded that exposure under real conditions is likely to be negligible, and the probability of transgene introgression is extremely small in most instances, with the exception of *B. rapa* in areas where it occurs close to oilseed rape. Transgene introgression is likely to take place when oilseed rape and *B. rapa* grow in close proximity over successive growing seasons, especially if no significant fitness costs are imposed on backcrossed plants by transgene acquisition (Snow et al., 1999). However, hybrids between *B. napus* and *B. rapa* are mostly triploid with low male fertility, and hence low ability to pollinate and form backcrosses with *B. napus* (Norris et al., 2004). The incidence of hybrids and backcrosses with *B. rapa* were found to be low in fields in Denmark (Jorgensen et al., 2004) and the United Kingdom (Norris et al., 2004). Recent observations in Canada confirmed the persistence of a glyphosate tolerance trait over a period of six years in a population of *B. rapa* in the absence of herbicide pressure (with the exception of possible exposure to glyphosate in one year) and in spite of fitness costs associated with hybridisation (Warwick et al., 2008). A single GM *B. rapa* × *B. napus* hybrid was also reported along a road in Vancouver (Yoshimura et al., 2006), confirming the potential for hybridisation between these two *Brassica* species, albeit at very low frequencies. However, Elling et al. (2009) measured the extent of hybridisation between autotetraploid *B. rapa* varieties (female) and *B. napus* (pollen donor) under experimental field conditions, and found that hybridisation with tetraploid *B. rapa* seemed to be more likely with diploid *B. rapa*. They reported that male fertility was higher in these hybrids than those formed with diploid *B. rapa* and suggested that introgression frequencies from *B. napus* to *B. rapa* would be higher in tetraploid *B. rapa*. They also reported the presence of some feral tetraploid *B. rapa* populations in northwest Germany, but did not report on interspecific hybrids or backcrosses in these populations.

Surveys and analyses conducted in Japan detected transgenes in seed collected from wild relatives (*B. rapa* and *B. juncea*) sampled at several ports and along roadsides and riverbanks in only two hybrid plants derived from a cross between *B. napus* and *B. rapa* (Saji et al., 2005; Aono et al., 2006, 2011). This confirms that the introgression of genetic material from feral oilseed rape to wild relatives, while theoretically possible, is very low due to the combined low conditional probabilities of spillage of GMHT oilseed rape in areas where wild relatives (e.g., *B. rapa*) are present, of germination given spillage, of survival of oilseed rape plants given germination, of hybridisation with its wild relatives given survival, and of the survival and the low fertility of interspecific hybrids themselves, which restrict backcrossing with the wild relative.

Glyphosate-based herbicides are frequently used for the control of vegetation along railway tracks and in arable land, open spaces, pavements or industrial sites (Monsanto, 2010). In these areas, the glyphosate tolerance trait is likely to increase the fitness of GMHT plants (be it feral plants or progeny from hybrids of oilseed rape and wild relatives) relative to non-glyphosate tolerant plants when exposed to glyphosate-based herbicides (Londo et al., 2010, 2011; Watrud et al., 2011). However, both the occurrence of feral GMHT oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape to wild relatives are likely to be low under an import scenario. Therefore, feral oilseed rape plants and genes introgressed into other cross-compatible plants would not create significant agronomic or environmental impacts, even after exposure to glyphosate-based herbicides.

Having reviewed all relevant scientific literature published since the adoption of its Scientific Opinion in 2004, the EFSA GMO Panel confirms that feral GMHT oilseed rape plants are likely to occur wherever GMHT oilseed rape is transported. However, as indicated in section 6.1.1.1, there is no evidence that the herbicide tolerance trait results in enhanced fitness, persistence or invasiveness of oilseed rape GT73, or hybridising wild relatives, unless those plants are exposed to glyphosate-based herbicides. Feral oilseed rape plants and genes introgressed into other cross-compatible plants would not create additional significant environmental impacts, even after exposure to glyphosate-based herbicides.

6.1.1.3. Potential interactions of the GM plant with target organisms[27]

Interactions of oilseed rape GT73 with target organisms are not considered an issue by the EFSA GMO Panel, as there are no target organisms.

6.1.1.4. Potential interactions of the GM plant with non-target organisms

Owing to the intended uses of oilseed rape GT73, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms are not considered an issue by the EFSA GMO Panel. Furthermore, there are no indications that the expression of the CP4 EPSPS protein in glyphosate tolerant crops will cause direct adverse effects on non-target organisms (CERA, 2010).

6.1.1.5. Potential interactions with the abiotic environment and biogeochemical cycles[28]

Owing to the intended uses of oilseed rape GT73, which exclude cultivation, and the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles are not considered an issue by the EFSA GMO Panel.

6.1.2. Post-market environmental monitoring[29]

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are: i) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; and ii) 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 environmental risk assessment. Monitoring is related to risk management, and the final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel has given its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2011).

The scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape GT73. As the scope of the application EFSA-GMO-NL-2010-87 does not include cultivation, the environmental risk assessment was concerned with the accidental release into the environment of viable seeds of oilseed rape GT73 during transport and processing for food uses, and with the horizontal gene transfer to bacteria occurring in the environment or human digestive tract. The environmental risk assessment identified no potential adverse effects to the environment. Therefore, no case-specific monitoring is necessary.

The general surveillance plan proposed by the applicant includes: i) the description of an approach involving operators (federations involved in oilseed rape import and processing) reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; ii) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and iii) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent period.

The EFSA GMO Panel considers that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of oilseed rape GT73, as the environmental risk assessment does not cover

[27] Technical dossier / Section D.8. and additional information received in September 2012.
[28] Technical dossier / Section D.10. and additional information received in September 2012.
[29] Technical dossier / Section D.11. and additional information received in April 2012.

cultivation and identified no potential adverse environmental effects. In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable seeds of oilseed rape GT73. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.2. Conclusion

Considering the intended uses of oilseed rape GT73, which exclude cultivation, the environmental risk assessment was concerned with the accidental release into the environment of viable seeds of oilseed rape GT73 during transport and processing for food uses, and with horizontal gene transfer to bacteria occurring in the environment or the human digestive tract. In the case of accidental release into the environment of viable oilseed rape GT73 seeds, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape GT73 plants, or hybridising relatives, unless those plants are exposed to glyphosate-based herbicides. The low levels of environmental exposure of oilseed rape GT73 plants indicate that the risk to non-target organisms is extremely low. Owing to the intended uses of oilseed rape GT73, the level of exposure of bacteria occurring in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape GT73 is expected to be low. Due to the bacterial origin of the CP4 *epsps* and *gox* genes and the activities of their encoded proteins, a highly unlikely but theoretically possible horizontal transfer of these recombinant genes in the background of natural variants of these genes and natural gene transfer processes between bacteria occurring in the environment, potentially adverse effects on human health or the environment are not expected. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of oilseed rape GT73 and the EFSA GMO Panel Scientific Opinions providing guidance on the post-market environmental monitoring of GM plants (EFSA, 2006b, 2011). In addition, the EFSA GMO Panel acknowledges the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable seeds of oilseed rape GT73. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of oilseed rape GT73 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of oilseed rape GT73 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 (with the exception of refined oil and food additives) in accordance with Regulation (EC) No 1829/2003. In evaluating oilseed rape GT73, the EFSA GMO Panel considered the information in the application EFSA-GMO-NL-2010-87, additional information provided by the applicant, scientific comments submitted by the Member States and relevant scientific publications. Further information was taken into account from previous applications for the placing on the market under EU regulatory procedures of oilseed rape GT73.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for oilseed rape GT73 are sufficient. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise any safety concerns. The levels of CP4 EPSPS and GOXv247 proteins in oilseed rape GT73 have been sufficiently analysed and the stability of the genetic modification has been demonstrated.

The comparative analysis indicated that no biologically relevant differences were identified in the compositional, agronomic and phenotypic characteristics of oilseed rape GT73 compared with its conventional counterpart, except for the newly expressed CP4 EPSPS and GOXv247 proteins.

Updates of the bioinformatic studies confirmed previous findings indicating no similarities of the CP4 EPSPS and GOXv247 proteins to known toxic proteins and allergens.

The EFSA GMO Panel concludes that considering the data available, no indication of potential concerns over the safety of the newly expressed CP4 EPSPS and GOXv247 proteins or the occurrence of unintended effects have been identified in either oilseed rape GT73 pollen/pollen-containing dietary supplements or the adventitious presence of trace levels of seeds in human foods. An equivalent assessment with isolated seed protein could not be made because of the lack of availability of relevant consumption and safety data.

The safety assessment identified no concerns regarding the potential allergenicity of oilseed rape GT73. In addition, several nutritional studies previously assessed by the EFSA GMO Panel confirmed that oilseed rape GT73 is as nutritious as its conventional counterpart (EFSA, 2004, 2009a).

As this application does not cover cultivation of oilseed rape GT73, there is no requirement for scientific information on the possible environmental effects associated with the cultivation of oilseed rape GT73. In the event of the accidental release into the environment of viable oilseed rape GT73 seeds during transport and processing for food uses, there are no indications of an increased likelihood of establishment and spread of feral oilseed rape GT73, unless those plants are exposed to glyphosate-based herbicides. Likewise, evidence indicates that hybridising wild relatives that may theoretically have acquired the herbicide tolerance trait through vertical gene flow are neither more likely to establish, nor more likely to spread than their non-GM comparators in the absence of glyphosate-based herbicides. Considering the intended uses of oilseed rape GT73, potential interactions of feral oilseed rape GT73 plants with the biotic and abiotic environment are not considered to be an issue due to the low levels of exposure. Owing to the intended uses of oilseed rape GT73, the level of exposure of bacteria occurring in the environment, including those in the gastrointestinal tract, to recombinant DNA from oilseed rape GT73 is expected to be low. The unlikely but theoretically possible transfer of the recombinant genes from oilseed rape GT73 to bacteria does not raise concerns. Furthermore, tolerance and resistance to glyphosate is widespread among bacteria occurring in the environment, making it unlikely that horizontal gene transfer would add to this natural background. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of oilseed rape GT73. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in its general surveillance plan.

While the EFSA GMO Panel is not in a position to conclude on the safety of oilseed rape pollen as such, it concludes that the genetic modification in oilseed rape GT73 does not constitute an additional health risk if oilseed rape GT73 pollen were to replace non-GM oilseed rape pollen. The environmental risk assessment of oilseed rape GT73 did not identify any safety concerns, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, received on 31 August 2010, concerning a request for the placing on the market of genetically modified oilseed rape GT73 submitted under Regulation (EC) No 1829/2003 by Monsanto.

2. Acknowledgement letter dated 27 September 2010 from EFSA to the Competent Authority of the Netherlands.

3. Letter from EFSA to applicant dated 17 November 2010 requesting additional information under completeness check.

4. Letter from applicant to EFSA received on 6 July 2011 providing additional information under completeness check.

5. Letter from EFSA to applicant dated 2 August 2011 requesting additional information under completeness check.

6. Letter from applicant to EFSA received on 31 October 2011 providing additional information under completeness check.

7. Letter from EFSA to applicant dated 22 November 2011 delivering the 'Statement of Validity' for application EFSA-GMO-NL-2010-87, regarding genetically modified oilseed rape GT73 submitted under Regulation (EC) No 1829/2003 by Monsanto.

8. Letter from EFSA to applicant dated 27 January 2012 requesting additional information and stopping the clock.

9. Letter from applicant to EFSA received on 16 March 2012 providing a timeline for submission of responses.

10. Letter from applicant to EFSA received on 23 April 2012 providing additional information.

11. Letter from EFSA to applicant dated 13 June 2012 requesting additional information and maintaining the clock stopped.

12. Letter from EFSA to applicant dated 5 July 2012 requesting additional information and maintaining the clock stopped.

13. Letter from applicant to EFSA received on 16 March 2012 providing a timeline for submission of responses.

14. Letter from applicant to EFSA received on 25 July 2012 changing the timeline for submission of responses.

15. Letter from applicant to EFSA received on 14 September 2012 providing additional information.

16. Letter from EFSA to applicant received on 13 December 2012 re-starting the clock.

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