

**Application EFSA-GMO-NL-2015-127 (maize 1507 x MON 810 x MIR162 x NK603)**  
**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

Country	Organization	Reference	Comment	GMO Panel response
Austria	Federal Ministry of Health	II.1 Hazard identification and characterisation	<p><b>I. GENERAL REMARKS</b></p> <p>The stacked event GM maize 1507xMON810xMIR162xNK603 expresses two cry proteins (Cry1Ab, Cry1F) and one vegetative insecticidal Bt protein (Vip3Aa20) that act to control certain lepidopteran insect pests. It further expresses the PAT protein, which confers tolerance to application of glufosinate-ammonium herbicide and was used as a selectable marker during transformation, and it expresses the CP4 EPSPS protein conferring glyphosate-tolerance to the maize plant.</p> <p>In this respect, the present notification of GM maize 1507xMON810xMIR162xNK603 is another example of a GM plant that expresses a combination of different insecticidal proteins derived from <i>B. thuringiensis</i>. The stacking of different Bt traits is a direct results of the development of resistant insect populations due to the introduction and cultivation of insect resistant GM plants.</p> <p>This development of Bt resistance in target pests is thought to threaten (not only the effectiveness of Bt technology in GM crops but also) the application of Bt-based pesticide sprays in agriculture. There is an example of a selected strain of <i>Ostrinia nubilalis</i> that was shown to have even developed more than 3000-fold resistance to Cry1F after 35 generations of selection and readily consumption of Cry1F expressing maize tissue (Pereira et al. 2007).</p> <p>Different genetic backgrounds:  The single event GM maize lines used in the breeding of the stacked event GM maize 1507xMON810xMIR162xNK603 are characterised by different genetic backgrounds. The use of these</p>	

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			<p>different lines in the breeding of the GM maize stack resulted in three F1 GM maize hybrids 1507xMON810xMIR162xNK603 differing in their genetic background (FROM CBI: Appendix B5 ). According to the breeding diagram presented by the applicant, all three F1 hybrids were used for commercial development (FROM CBI: Appendix B5 ).</p> <p>With exception of the evaluation of the generational stability of the GM trait, for which data derived from different genetic backgrounds provide additional value, we would appreciate if all relevant data are established for one particular GM line in order to ensure comparability and consistency among the data presented. Thus, we request clarification regarding the use of different hybrid lines, and with regard to hybrid C the notifier should submit data on the expression of the inserts derived from this hybrid.</p> <p>[Appendix B5, Breeding tree. Dossier EFSA/GMO/NL/2015/127.</p> <p>Pereira EJG, Lang BA, Storer NP, Siegfried BD, 2007. Selection for CryIF resistance in the European corn borer and cross-resistance to other Cry toxins. Oxford, ROYAUME-UNI, Blackwell.]</p>	<p>The data used for the risk assessment was generated according to Regulation (EU) No 503/2013 and EFSA Guidance on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011) and is considered adequate.</p>
Austria	Federal Ministry of Health	II.1.2.1 Information relating to the genetic modification	<p>1.2.1.3 Source of nucleic acid(s) used for transformation, size, and intended function of each constituent fragment of the region intended for insertion</p> <p>Scientific Information, p. 26:  The applicant maintains that "the transgenic proteins expressed in 1507xMON810xMIR162xNK603 maize have a history of safe consumption as Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been a part of the food supply."  We would like to indicate that there has been no specific monitoring systems established which would have the power to reveal adverse effects of these</p>	<p>The GMO Panel takes note of the comment. (section 3.4.3.3 of the scientific opinion).</p>

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			<p>transgenic proteins on human health from an epidemiological perspective nor have there been epidemiological studies which would have evaluated respective potential adverse effects scientifically. There is no scientific (i.e. supported by epidemiological data) evidence on the human population level available in favor of or against the safety of these transgenic proteins. Therefore this statement of the applicant is an assumption not backed up by scientific evidence. We would like to ask the EFSA GMO Panel to take this observation into consideration for their evaluations.</p> <p>Scientific Information, p. 28:  The applicant maintains, "Although S. viridochromogenes has not been used as a food source, it might be present in food unintentionally " in support of a history of safe use of the derived PAT protein. We would like to indicate that this line of argumentation (unintentional presence in food -&gt; safe product) is inadequate for a serious risk assessment and not eligible as scientific evidence in favor of the safety of a product. We would like to ask the EFSA GMO Panel to take this observation into consideration for their evaluations.</p>	<p>The GMO Panel takes note of the comment. (section 3.4.3.3 of the scientific opinion).</p>
Austria	Federal Ministry of Health	II.1.2.2 Information relating to the genetically modified plant	<p>2.2.2 Information on the sequences actually inserted or deleted</p> <p>Scientific Information, p. 34 (1507 maize):  The applicant maintains that "1507 maize contains an almost full-length copy of the DNA insert ... without internal rearrangements", and that "both cry1F and pat gene cassettes are intact within the transgenic event and the DNA sequences of the genes are identical to those in the original plasmid."  We would like to indicate that the transgenic insert actually present in maize 1507 is affected by substantial rearrangements (i.e. fragmentation and duplication of the cry1F gene, duplication and inversion of 2 pat fragments 5' ahead and inverse integration of a pat gene fragment 3' downstream of</p>	<p>The organisation of each insert has been assessed previously in the framework of the risk assessment of each single event and is reported in the respective scientific opinions. The structure of the events was maintained in this stack.</p>

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		<p>the PHI8999A cassette; see figure 2). We would like to ask the EFSA GMO Panel to take this observation into consideration for their evaluations.</p> <p>Scientific Information, p. 34 (MON810 maize):  The applicant does not indicate that the transgenic insert in MON810 has been affected by a deletion of the NOS-terminator in the 3' region of the transformation cassette and that the transgenic maize variety does not produce the wild type Cry1Ab toxin but a fusion protein derived from a run off transcript containing two or eighteen additional C-terminal amino acids. (Levine 2004; Rosati et al. 2008). We would like the EFSA GMO Panel to take note of this observation.</p> <p>Scientific Information, p. 35:  The applicant maintains that "a detailed molecular analysis has been conducted to confirm that the copy number, structure and organisation of the respective individual inserts in 1507xMON810xMIR162xNK603 maize are equivalent to those in 1507, MON810, MIR162 and NK603 maize."  The referred study report (Annex 10_PHI-2011-140 presented in support of this statement contains the description of the applied Southern blot procedures and of an event-specific PCR. The Southern blot results for maize 1507, MIR162, and NK603 do not support the integrity of the transgenic insert (for details see the discussion on each event below). The presented event-specific PCRs indicate the presence of the expected insertion site but do not provide proof of evidence for the intactness of the whole transgenic insert in the genomic DNA of the stacked maize variety (1507xMON810xMIR162xNK603) under investigation. We would like the EFSA GMO Panel to take note of these observations.</p> <p>Scientific Information, p. 37-38 (Fig. 3):  The applicant maintains that "NcoI digested DNA resulted in a consistent band of approximately 3600 bp</p>	<p>The GMO Panel takes note of the comment.</p> <p>The GMO Panel takes note of the comment.</p> <p>In addition to the Southern analyses presented, the integrity of the inserts has been analysed by sequencing data provided upon EFSA's request (additional information clock2, 03/05/2016), and according to the Regulation (EU) No 503/2013 and the EFSA guidance for the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011), for all the individual events in the stack.</p> <p>The GMO Panel considers that the quality and results of the whole set of Southern analyses and sequencing data presented are considered sufficient to conclude on the maintenance of the structure of the inserts of the single events in the stack.</p> <p>The conclusions are not based on the outcome of one Southern analysis but on consideration of data from all different combinations of probe-restriction enzymes used to show the presence and maintenance of structure of the 4 events.</p>
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		<p>in the 1507xMON810xMIR162xNK603 maize and the 1507 maize samples."</p> <p>This is not correct. The bands in lanes 7 and 8 (figure 3; samples T5 and T6 of the stacked event) are significantly below the fragments of the control comparator (1507; single event) and below the fragment of the T3 sample of 1507xMON810xMIR162xNK603. The supplied Southern blot analysis is therefore not adequate to provide evidence for the intactness of the insert.</p> <p>It is remarkable that the applicant selected a restriction enzyme which generates two different fragments of 3576 and 3611 bp (see Figure 2, p. 37) which apparently cannot be separated by the applied agarose gel electrophoresis and overlap as a single band on the Southern blot. The applied procedure, thus, would not even detect a DNA segment deletion/insertion of 35 bp in the transgenic insert. The applicant is requested to provide a new Southern blot displaying all 5 reference bands representing the intact insert in a straight line on the blot. The applicant is requested to provide a restriction enzyme - probe combination which results in clearly separable and distinct fragments on the blot which do not overlap.</p> <p>Scientific Information, p. 39 (Fig. 4):  The bands which should represent the expected 3.6 kb fragment are on completely different positions if lane 5 is compared to lanes 7 and 8. This discrepancy in the banding pattern is especially pertinent considering that the &gt; 8,6 kb fragment is more or less straight in line with all samples tested (lanes 5-10). This Southern blot is not eligible to provide proof of evidence for an intact transgenic insert in the stacked event. We would like the EFSA GMO Panel to take note of this observation.</p> <p>Scientific Information, p. 43 (Fig. 8):  The bands in lanes 6, 7 and 8 representing samples T4-T6 of the stacked event are located significantly</p>	
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		<p>below the corresponding band of sample T3 (= lane 5). The supplied Southern blot analysis is therefore not adequate to provide evidence for the intactness of the insert. We would like the EFSA GMO Panel to take note of this observation.</p> <p>The discrepant position of the fragments derived from the stacked event (T3, T4, T5, T6) is remarkable for the Southern blots represented in Figures 3, 4, 8 and 9, but not for the Southern blot using the MON810 specific probe in figure 6. We would like the EFSA GMO Panel to take note of this observation.</p> <p>[Levine EB, 2004. Corn event MON810 and compositions and methods for detection thereof. Monsanto Technology LLC. US Patent No. 6,713,259.</p> <p>Rosati A, Bogani P, Santarlasci A, Buiatti M, 2008. Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. Plant Mol Biol 67(3): 271-281.]</p>	
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Austria	Federal Ministry of Health	II.1.2.2 Information relating to the genetically modified plant	<p>1.2.2.3 Information on the expression of the inserted/modified sequence</p> <p>The applicant presents data for the concentrations of Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins from various plant tissues (grain and forage) originating from field trials conducted in the US in 2012 at 4 locations (FROM CBI: Annex 20). ELISA results are presented for the GM maize stack treated with conventional as well as with the intended herbicide treatment.</p> <p>The applicant states that the trial sites were "selected on the basis of their inclusion in the commercial maize-growing regions of North-America " and "considering geographic distribution to represent a diversity of environmental conditions " (Scientific Information, p. 61). However, no data are provided to substantiate this statement (see also our comments to 1.3). The statistical analysis is restricted to basic descriptive statistics, such as means, data ranges and standard deviations and lacks an analysis of variance. Thus potential interactions with environmental conditions or the applied herbicides (genotype x environment interactions) are not accounted for. In addition, the use of expression data from four sites only may not be sufficient to adequately establish the range of variation of expression of transgenic gene products in the stacked GM maize (cf. Trtikova et al. 2015).</p> <p>The assessment of variation of expression however is important in order to estimate the maximum levels of expression of the transgenic constituents under representative growing conditions. This maximum expression needs to be known in order to be able to assess exposure of humans, animals and the environment via consumption of food or feed products produced from GM maize.</p> <p>Furthermore, the applicant concludes on a lack of potential interaction between the stacked inserts at the level of gene transcription or translation arguing that the expression of the proteins in the stacked GM maize 'appears similar' to those in the respective single</p>	<p>The data on protein expression provided are in agreement with EFSA guidance on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011). Expression data of each of the inserts has been analysed in detail in the framework of previous applications for the commercialization of the single events. In the framework of this application the objective of the expression analyses is 1) to assess any changes of expression with respect to the previously assessed single events that may indicate unintended interactions between the different inserts and 2) to provide dietary exposure estimations. The data provided are sufficient to demonstrate that the ranges in protein expression levels observed are comparable between the single events and the events when stacked by conventional crossing.</p> <p>The findings in the article published by Trtikova et al (2015) have been evaluated by EFSA previously (EFSA, 2015).</p> <p>EFSA (European Food Safety Authority), 2015. Relevance of a new scientific publication (Trtikova et al., 2015) on previous EFSA GMO Panel conclusions on the risk assessment of maize MON 810 and other Cry1Ab-expressing Bt-maize events. EFSA supporting publication 2015:EN-878. 11 pp.</p>
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		<p>events (Scientific Information, p. 91). Again no detailed statistical analysis of the expression data is presented to support this argument. Any significant difference in expression however may also indicate interactions between the stacked inserts. Such epigenetic interactions between the two single events cannot be excluded and justify a thorough assessment of the reliability of expression (Dietz-Pfeilstetter 2010). Thus, the applicant is requested to provide a justification that the expression data are representative for GM maize stack 1507xMON810xMIR162xNK603 produced during commercial cultivation and to provide a detailed statistical analysis (i.e. analysis of variance) of expression data, which constitutes an additional value for the exposure and the toxicological assessment.</p> <p>[Annex 20, Expressed trait protein concentration of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: U.S. and Canada test sites (including ELISA method validation summary reports). Dossier EFSA/GMO/NL/2015/127.</p> <p>Dietz-Pfeilstetter A, 2010. Stability of transgene expression as a challenge for genetic engineering. Plant Sci 179(3): 164-167.</p> <p>Trtikova M, Wikmark OG, Zemp N, Widmer A, Hilbeck A, 2015. Transgene expression and Bt protein content in transgenic Bt maize (MON810) under optimal and stressful environmental conditions. PLoS One 10(4): e0123011.]</p>	
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Austria	Federal Ministry of Health	II.1.2.2 Information relating to the genetically modified plant	<p>1.2.2.2 Information on the sequences actually inserted or deleted</p> <p>The data submitted for molecular characterisation of GM maize 1507xMON810xMIR162xNK603 consist of Southern blots to demonstrate the presence of the introduced traits (Cry1F, PAT, Cry1Ab, Vip3Aa20 and CP4 EPSPS) by comparison with the respective parental single events. The Scientific Information furthermore refers to data submitted for the molecular characterisation of the parental single events as well as the information submitted for a number of previously assessed sub-combinations of events.</p> <p>The identity of the transgenic inserts contained in GM maize 1507xMON810xMIR162xNK603 is not demonstrated by comprehensive experimental data. The submitted Southern blot data permit only a very coarse assessment of the integrity of the individual transgenic inserts in GM maize 1507xMON810xMIR162xNK603 at best. No assessment of all parts of the transgenic inserts present in the stacked event is presented for a detailed comparison with the respective inserts in the parental events. It is therefore requested that the notifier submits a more comprehensive characterisation of the inserts to assess the identity with the transgenic inserts present in the parental events.</p> <p>The notifier should further address any remaining issues, which were identified in our comments on assessment of the respective parental events (GM maize lines 1507, MON810, MIR162 and NK603) and which were not addressed in full since.</p> <p>In addition, the notifier should include all information in the Scientific Information which is relevant for assessment, e.g. further information on the probes used to detect the e35S-promoter sequences and cry1Ab, as well as CP4 EPSPS coding sequences</p>	<p>In addition to the Southern analyses presented, the integrity of the inserts has been analysed by sequencing data provided upon EFSA's request (additional information clock2, 03/05/2016), and according to the Regulation (EU) No 503/2013 and the EFSA guidance for the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011), for all the individual events in the stack.</p> <p>The GMO Panel considers that the quality and results of the whole set of Southern analyses and sequencing data presented are considered sufficient to conclude on the maintenance of the structure of the inserts of the single events in the stack.</p>
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		<p>originating from GM maize MON810 and GM maize NK603. The respective information is not included in the Scientific Information (see FROM CBI: Annex 10 , p. 20).</p> <p>The design of the Southern blot experiments chosen by the notifier should facilitate that the respective results are fully conclusive as submitted in the Scientific Information. Diagnostic bands from the Southern blot fingerprints should be easily discernible in length and clearly visible on the Blots. Some results submitted for characterisation of the transgenic inserts are not adequate in this respect. As indicated by the notifier, e.g. bands detected with the cryF1 probe in GM maize 1507xMON810xMIR162xNK603 are difficult to distinguish due to their slight differences in size (3.5 and 3.6 kb) and the differences in signal strength (see Scientific Information Fig. 3, p. 38, and FROM CBI: Annex 10 , p. 32). Experiments for demonstrating structural integrity of Vip3Aa20 detected fragments of 7.0-8.0 kb. Fragments of such length are not easily discernible, and thus cannot be unambiguously identified by the used experimental design.</p> <p>The notifier should therefore reconsider his strategy for molecular characterisation and submit experimental results that are fully conclusive.</p> <p>[Annex 10, Genetic stability and equivalency of DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6 maize using Southern blot analysis and event-specific polymerase chain reaction. Dossier EFSA/GMO/NL/2015/127.]</p>	
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Austria	Federal Ministry of Health	II.1.2.2 Information relating to the genetically modified plant	<p>1.2.2.4 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant</p> <p>The genetic stability of GM maize 1507xMON810xMIR162xNK603 was not assessed sufficiently by the notifier. Contrary to the statement of the notifier that "Southern blot analysis was performed on a representative set of 1507xMON810xMIR162xNK603 maize plants in order to confirm the genetic stability of the insertions after crossing " (FROM CBI: Annex 10 , p. 32-37), only 4 individual plants were subjected to a Southern blot analysis, which furthermore does not allow for a detailed assessment of stability of all insert parts. This is deemed insufficient given that the transgenic inserts derived from different parental events share some homologous sequences (e.g. 35S promoter elements) which might generate instability during propagation. The notifier should submit additional information to address the issue. Specifically, an adequate number of individual plants should be analysed with methods which allow for a more detailed assessment of the integrity of the transgenic insertions and the flanking sequences. The PCR analysis of 65 plants (FROM CBI: Annex 10 , Table 2, p. 23) submitted by the notifier together with the Southern blot analysis cannot be regarded as appropriate in this respect. Furthermore, the level of stability which can be detected by these experiments should be indicated by the notifier.</p> <p>[Annex 10, Genetic stability and equivalency of DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6 maize using Southern blot analysis and event-specific polymerase chain reaction. Dossier EFSA/GMO/NL/2015/127.]</p>	<p>In addition to the Southern analyses presented, the integrity of the inserts has been analysed by sequencing data provided upon EFSA's request (additional information clock2, 03/05/2016), and according to the Regulation (EU) No 503/2013 and the EFSA guidance for the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011), for all the individual events in the stack.</p> <p>The GMO Panel considers that the quality and results of the whole set of Southern analyses and sequencing data presented are considered sufficient to conclude on the maintenance of the structure of the inserts of the single events in the stack.</p>
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Austria	Federal Ministry of Health	II.1.3 Comparative analysis	<p>The data presented for the comparative analysis were generated in field trials conducted in North America (12 sites in 2012) which included plots with conventional and intended (glyphosate and glufosinate) herbicide treatment. The assessment comprised a compositional analysis (conducted only at 8 primary sites) and agronomic &amp; phenotypic characteristics conducted at all 12 sites (FROM CBI: Annex 21 ; Annex 22 ; Annex 23 ). In addition, a study evaluating seed germination of the stacked GM maize was presented (FROM CBI: Annex 28 ).</p> <p>The applicant states that the trial sites were "selected on the basis of their inclusion in the commercial maize-growing regions of North-America " and "considering geographic distribution to represent a diversity of environmental conditions " (Scientific Information, p. 61). However, no data (e.g. characterisation of the test sites regarding typical local agronomic practices or prevailing pest and disease pressure or other biotic or abiotic stresses) are provided substantiating this statement.</p> <p>However, the EFSA guidance documents (EFSA 2010; EFSA 2015) as well as Implementing Regulation (EU) No 503/2013 (EC 2013) state that a justification shall be provided that the sites and conditions are representative of the range of receiving environments, where the crop will be commercially grown, explicitly justifying the choice of sites (EFSA 2010). Thus, we request that the applicant provides information on the above mentioned aspects.</p> <p>[Annex 21, Agronomic characteristics of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: U.S. and Canada test sites (EU study format). Dossier EFSA/GMO/NL/2015/127.</p> <p>Annex 22, Nutrient composition of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: U.S.</p>	<p>The field trials were conducted in 2012 and 2015 in typical maize growing areas of North America, representing regions of diverse agronomic practices and environmental conditions, which is supported by the geographic map indicating the locations, the information provided on the variety of agronomic practice, soils and meteorological factors. In order to improve the representativeness of the selected field trials, EFSA published a guidance document on the agronomic and phenotypic characterisation of genetically modified plants (EFSA GMO Panel, 2015a). Application EFSA-GMO-NL-2015-127 was submitted during the transitional period of the GMO Panel guidance. Therefore, the requirements of the guidance document were not fully applicable for this application. Spontaneous information to integrate the selection of sites with new field trials conducted in 2015 were provided on 30/5/2016 and 06/06/2016. The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and management practices of the field trial sites are acceptable for receiving environments where the tested materials could be grown.</p>
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		<p>and Canada test sites (EU study format). Dossier EFSA/GMO/NL/2015/127.</p> <p>Annex 23, Biological relevance of statistically observed nutrient differences between maize grain containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6 and control maize grain from PHI-2012-023/020. Dossier EFSA/GMO/NL/2015/127.</p> <p>Annex 28, Evaluation of germination and viability of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: Controlled environment test site. Dossier EFSA/GMO/NL/2015/127.</p> <p>EC, 2013. Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48.</p> <p>EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111.</p> <p>EFSA, 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. The EFSA Journal 13(6):4128: 1-44.]</p>	
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Austria	Federal Ministry of Health	II.1.3.1 Choice of the conventional counterpart and additional comparators	<p>Use of three different GM hybrids:</p> <p>The notifier presents the breeding history of GM maize 1507xMON810xMIR162xNK603 in FROM CBI: Appendix B5 (Figure 1 and Table 1).</p> <p>The diagram and Table 1 show maize hybrid seed lots with three different genetic backgrounds are used in the risk assessment with the assignment of the seed lots being as follows:</p> <ul style="list-style-type: none"> <li>i) PHE4N/PHHHN seed - molecular characterisation</li> <li>ii) PHE4N/PHH9H seed - comparative assessment and protein expression analysis</li> <li>iii) SYNTAX5707/PH581 seed - 42-day feeding study in broilers</li> </ul> <p>There are remaining questions as regards the rationale behind the notifier's decision for using these three different hybrids:</p> <p>In an optimal test set up for GM risk assessment one GM maize hybrid is used in all different steps of the risk assessment process, and so the results of the different tests would complement one another. The combined tests (molecular characterisation, comparative assessment, toxicology and immunology, nutritional assessment) would provide a holistic picture of the GM maize under investigation enabling a robust evaluation whether the GM maize 1507xMON810xMIR162xNK603 and its inherent properties may (or may not) give rise to concern.</p> <p>In particular, if the 42-day feeding study in broilers for nutritional assessment were performed with GM maize of the same genetic background as used in compositional assessment, the results of the broiler study would help support the notifier's reasoning that - in spite of the high number of significances (82.5%) and trends - nutritional equivalence with conventional maize still could be demonstrated.</p> <p>The use of GM varieties with different genetic backgrounds in GM risk assessment steps introduces uncertainty and is not warranted unless there are substantial reasons for doing so.</p> <p>The notifier is requested to present such reasoning.</p>	<p>The EFSA GMO Panel thanks Austria for the comment. The use of the same genetic background for the different studies is not a requirement and the 3 reported studies are not linked. What is considered essential is the selection of the appropriate comparator in each experiment. In case of application EFSA-GMO-NL-2015-127 for the comparative analysis, two independent sets of field trials were received: North America (2012) and North America (2015); both sets were considered, although for different purposes. Compositional data were provided only for the field trials in 2012. Agronomic-phenotypic data were measured in both sets, but they were assessed based only on data from the field trials in 2015, as those in 2012 did not include the measurement of yield components (yield and kernel weight) and therefore were considered incomplete. The 2015 field trials report and data were spontaneously received on the 30/5 and 6/6 2016.</p> <p>The nutritional assessment of the GM crop was based on the results of compositional analysis (section 3.4.3.6 of the Scientific opinion), and differences in compo endpoints did not show nutritional concerns. Therefore, further animal studies are not necessary. However, the GMO Panel did not observe adverse effects in the broiler study provided (appendix B in the Scientific opinion).</p>
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			<p>Breeding scheme:  The breeding scheme of the conventional counterpart is not part of the notification and should be provided. According to EFSA guidance, "The applicant should provide information on the breeding scheme (pedigree) in relation to the GM plant, the conventional counterpart and/or other comparator(s) used in the risk assessment together with a clear justification for their selection."  Only if the information on various steps of selfing and (back)crossing which have taken place in the development of the GM plant and the conventional counterpart are presented, the similarity of the genetic background and the equivalence of the non-GM plants used as a comparator, can be assessed.  Furthermore, it is not clear how the notifier arrives at the conclusion that "the conventional counterpart shares greater than 94% genetic similarity " (Scientific Information, p. 62).  The underlying calculations of the value (94%) are important criteria for the validity of the comparative assessment and should be presented.</p> <p>[Appendix B5, Breeding tree. Dossier EFSA/GMO/NL/2015/127.]</p>	<p>The breeding tree of the conventional counterpart is reported in table 1 of Appendix B5 (CI). The EFSA GMO Panel requested further information to better characterise the genetic similarity between the tested GM line and the conventional counterpart. This information was received on the 29/16/2016.</p>
Austria	Federal Ministry of Health	II.1.3.4 Comparative analysis of composition	<p>Simulation analysis (Scientific Information, p. 67):  A simulation analysis was conducted resulting in an estimated average number of significant differences between the GM maize and conventional counterpart of 50. This additional test is said to give an explanation for the high number of observed differences between the GM maize and its conventional counterpart (Scientific Information, p. 73): "The simulation results indicated the estimated average number of significant differences between CHT 1507xMON810xMIR162xNK603 maize and control maize was 50 with the 2.5th and 97.5th percentiles being 43 and 56, respectively; and the estimated</p>	<p>The simulation study was performed following the indications of EFSA GMO Panel (2010), as confirmed by the software code used for the simulation and included by the applicant in the submission. The procedure allowed for the same variation between the GMO and the comparator as between the mean of the reference varieties. The result of such procedure is the number of significant differences that would be expected between two randomly chosen reference varieties. This is considered as the 'degree of acceptable difference' between the GMO and the comparator and can be used as 'a useful point of reference for judging the actually obtained number of significant differences between GMO and conventional counterpart' (EFSA GMO Panel, 2010). There is no direct relation between the expected number of significant differences (which is calculated</p>

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		<p>average number of significant differences between IHT 1507xMON810xMIR162xNK603 maize and control maize was 50 with the 2.5th and 97.5th percentiles being 42 and 57, respectively. The observed numbers of significant differences (40 between CHT 1507xMON810xMIR162xNK603 maize and the control maize, and 40 between IHT 1507xMON810xMIR162xNK603 maize and the control maize) are below the expected ranges, as suggested by the simulation results, and therefore, are not a source of concern."</p> <p>Relating to simulation analysis, EFSA (2010) notes, "In a somewhat more formal analysis it can be investigated, e.g. by simulation studies, how many significant results can be expected under the null hypothesis of GMO and conventional counterpart being equivalent varieties (that is, allowing for the same variation as found between commercial varieties)." Thus, the notifier should make clear if this "more formal" approach was applied in simulation analysis assuming the same variation between GMO and conventional counterpart as between commercial varieties.</p> <p>Another point is that EFSA notes, "Simulation can be used to estimate how likely it is to obtain the actually observed number of significant results under the assumption that GMO and conventional counterpart means might in fact be slightly different, given a distribution of acceptable differences. In these simulations the degree of acceptable difference should be specified, and that can for example be taken equal to the observed variation between the means of the commercial reference varieties."</p> <p>In this respect, the notifier is requested to specify the "degree of acceptable difference" used in the simulation analysis.</p> <p>The notifier is further requested to provide a justification for the assumption that the estimated average number of significant differences between GM maize and conventional counterpart is 50, which is in</p>	<p>between two randomly chosen reference varieties) and the degree of genetic similarity between the GMO and the comparator.</p>
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		<p>contrast to the statement that the GM maize and the conventional counterpart share greater than 94% genetic similarity (cf. Scientific Information, p. 62 and 73).</p> <p>EFSA proposes its statistical model for comparative assessment concluding:</p> <p>"It is a consequence of the simplified graphical display that confidence limits for the difference test were chosen as 90%, yielding a 10% size for the difference test, in which 1 in 10 of such tests is expected to yield a significant result by chance alone " (EFSA 2011, p. 17).</p> <p>Since the notifier argues that "the statistical model was adopted directly from EFSA Guidance" (Scientific Information, p. 66), we think that there should be no contradiction between the number of significant results to be expected by chance alone between EFSA and the notifier.</p> <p>[EFSA, 2010. Scientific opinion of the GMO Panel on statistical considerations for the safety evaluation of GMOs. The EFSA Journal 8(1):1250: 1-59.</p> <p>EFSA, 2011. Guidance of the GMO Panel for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5):2150: 1-37.]</p>	
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Austria	Federal Ministry of Health	II.1.3.4 Comparative analysis of composition	<p>Specific remarks in relation to significant differences:  The compositional analysis of GM maize 1507xMON810xMIR162xNK603 was performed with data derived from 8 field trials in North-America. 71 analytes were measured and used for a comparison between the stacked GM maize and the non-GM conventional counterpart (difference test).  Two comparisons were carried out:  i) The conventional counterpart (variety PHE4NxPHH9H) was compared against the conventional herbicide treated (CHT) GM maize. In the CHT comparison, 40 of the 71 analytes were found to be significantly statistically different.  ii) The conventional counterpart (variety PHE4NxPHH9H) was compared against the intended herbicide treated (IHT) GM maize. In the IHT comparison, also 40 of the 71 analytes were found to be significantly statistically different.</p> <p>All significant differences concerned outcome type 2 and type 4 analytes according to the classification by EFSA (2010). Although type 2 analytes (per definition) lie within the equivalence limits, which indicates equivalence to the set of commercial varieties, significances of difference tests are considered reason enough to further examine each analyte/endpoint. Many type 2 analytes also showed trends (e.g. grain proximates). (For trends, please see comment below.) It is said by EFSA Guidance that "outcome types 1 or 2 may easily be obtained for characteristics that are stable and precisely measured within each genotype, but that have a large natural variation among commercial genotypes " (EFSA 2010). Hence, the notifier should prove if this is the case with the observed type 2 analytes.</p> <p>Trends in significant differences:  82.5% of the significantly different analytes (33) are confirmed by trends in across-site analysis between CHT and IHT comparisons; that is almost 50% of all</p>	<p>The GMO Panel assessed all the significant differences between maize 1507 x MON810 x MIR162 x NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the three-event stack maize and the non-GM comparator and falling under category III/IV were identified.</p>
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		<p>71 analytes measured.</p> <p>This is considered to be a relatively high number which gives an indication of inherent, constant differences between the GM maize 1507xMON810xMIR162xNK603 and its conventional counterpart due to the genetic modification.</p> <p>Following a list of analytes showing trends for significant differences:</p> <p>1) All eight proximates show trends in grain:</p> <ul style="list-style-type: none"> <li>- Moisture, protein, fat, fibre, ADF, NDF, ash were significantly (higher in the GM maize)</li> <li>- carbohydrates was significantly (lower)</li> </ul> <p>2) NDF shows a trend in forage (higher)</p> <p>3) 6 of 12 fatty acids show trends in grain:</p> <ul style="list-style-type: none"> <li>- palmitic acid, palmitoleic acid, stearic acid, linolenic acid (higher)</li> <li>- oleic acid, eicosenoic acid (lower)</li> </ul> <p>4) 9 Amino acids show trends in grain:</p> <ul style="list-style-type: none"> <li>- alanine, aspartic acid, glutamic acid, isoleucine, leucine, phenylalanine, serine, threonine, valine (higher)</li> </ul> <p>5) 4 minerals show trends in grain:</p> <ul style="list-style-type: none"> <li>- phosphorus, iron, copper, potassium (higher)</li> </ul> <p>6) 2 Vitamins show trends in grain:</p> <ul style="list-style-type: none"> <li>- pyridoxine, γ-tocopherol (higher)</li> </ul> <p>7) 3 secondary metabolites/anti-nutrients show trends in grain:</p> <ul style="list-style-type: none"> <li>- coumaric acid, ferulic acid, phytic acid (higher)</li> </ul> <p>The proximate analysis of the notifier (Scientific Information, p. 74) concerns only one endpoint (total fat) but other seven proximates also with trends are not analysed. This is the same with most of the other listed endpoints.</p> <p>Although significant differences or trends do not mean a risk per se, a large number of significances gives rise to concerns that genetic modification has resulted in unintended effects that might have the potential to be harmful. It should be especially recognised that health risk associated with changed pattern of minor metabolites or secondary metabolites (e.g. plant</p>	
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			<p>hormones) may not be verifiable during compositional analysis because evaluation is limited to the 84 compounds included in the assessment according to OECD consensus documents.</p> <p>Moreover, as data are available for single events, it was ideal when the data of the stacked GM maize 1507xMON810xMIR162xNK603 were directly compared with those data. This would be a logical addition to the assessment of stacked GM maize, particularly because the notifier uses the single event results for proving the safety of the stacked event (cf. Scientific Notification, p. 61).</p> <p>EFSA Guidance on stacked events supports the need to focus on "potential interactions between the events", which particularly could be assessed by such a direct comparison.</p> <p>[EFSA, 2010. Scientific opinion of the GMO Panel on statistical considerations for the safety evaluation of GMOs. The EFSA Journal 8(1):1250: 1-59.]</p>	
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Austria	Federal Ministry of Health	II.1.3.4 Comparative analysis of composition	<p>The scope of the comparative analysis concerning food and feed risk assessment is considered too narrow with a view to the characteristics of GM stacked maize 1507xMON810xMIR162xNK603. As the GM maize stack is designed for the use of the complementary herbicides, i.e. glyphosate and glufosinate, the residual levels as well as residual metabolites of these herbicides also need to be subject to analysis.</p> <p>We therefore request that the applicant presents a broader data basis with respect to the compositional analysis and includes the analysis of residual glyphosate and glufosinate and their metabolites in his compositional assessment.</p> <p>A recent review of compositional analyses and feeding studies conducted with herbicide tolerant crop material demonstrated the need to better take into account current production conditions for herbicide-tolerant crops in the design of field tests (Cuhra 2015). This is necessary to ensure that assessments are representative of commercial cultivation conditions.</p> <p>Due to increasing weed resistance to glyphosate application rates and frequencies of application of glyphosate-herbicides are rising (Heap 2015; Benbrook 2016). This seems to be reflected in the fact that two applications of the intended herbicides have been applied in the field trials conducted for the comparative assessment while only one application of a conventional herbicide mix was used as conventional herbicide treatment (FROM CBI: Annex 21 ). The more frequent use and/or higher amounts of herbicides used in commercial cultures may lead to higher levels of herbicide residues and metabolites in harvested crop material (cf. Benbrook 2012; Benbrook 2016; Myers et al. 2016).</p> <p>[Annex 21, Agronomic characteristics of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: U.S. and Canada test sites (EU study format). Dossier</p>	The risk assessment of the herbicides and their metabolites is outside the remit of the GMO Panel.
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			<p>EFSA/GMO/NL/2015/127.</p> <p>Benbrook C, 2012. Impacts of genetically engineered crops on pesticide use in the U.S. - the first sixteen years. Environmental Sciences Europe 24(1): 24.</p> <p>Benbrook CM, 2016. Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28(1): 1-15.</p> <p>Cuhra M, 2015. Review of GMO safety assessment studies: glyphosate residues in Roundup Ready crops is an ignored issue. Environmental Sciences Europe 27(1): 1-14.</p> <p>Heap I, 2015. The International Survey of Herbicide Resistant Weeds; <a href="http://www.weedscience.org">www.weedscience.org</a>; (last accessed: 04/11/2015).</p> <p>Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, Hansen M, Landrigan PJ, Lanphear BP, Mesnage R, Vandenberg LN, Vom Saal FS, Welshons WV, Benbrook CM, 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ Health 15(1): 19.]</p>	
Austria	Federal Ministry of Health	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	<p>Evaluation of germination and viability (FROM CBI: Annex 28 ):</p> <p>Laboratory tests on germination (warm, cold and diurnal germination tests) were carried out. The GM maize 1507xMON810xMIR162xNK603 was tested in comparison to the non-GM conventional counterpart and two conventional maize varieties.</p> <p>i) Materials:</p> <ul style="list-style-type: none"> <li>• Test design is very poor; only two additional reference varieties were included in the test.</li> <li>• It is NOT described where the seed came from (numbers of seed lots are lacking, size of seed lot is not described), if all the lines were grown on the same site/ different sites or under equal climatic and</li> </ul>	<p>Seeds of GM maize 1507 × MON810 × MIR162 × NK603 and the conventional comparator used in the 2012 and 2015 field trials were produced from plants harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their purity via event specific quantitative polymerase chain reaction analysis. A statistically significant reduction was observed between mean germination rates of maize 1507 × MON810 × MIR162 × NK603 and control maize only under warm growing conditions. The reduction in germination rate did not alter the suitability of the materials for the comparative analysis.</p>

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			<p>whether conditions (influences on germination!)</p> <p>ii) Methods: Experimental design and Germination Tests</p> <ul style="list-style-type: none"> <li>• The "pure seed definition" is lacking</li> <li>• Cold germination test is no ISTA-method</li> <li>• Diurnal germination test is no ISTA-method</li> <li>• Evaluation: Only germinated seed and non-germinated seed were counted, but no differentiation is made on normal and abnormal germinated seed</li> <li>• There is no reference made to ISTA/AOSA, which seems to be essential in the case of seed testing on germination (see References, page 15)</li> </ul> <p>iii) Conclusion:</p> <p>The report concludes: "A statistically significant difference was observed between mean germination rates of 1507xMON810xMIR162xNK603 maize (94.5%) and control maize (98.5%) under warm growing conditions; however, both mean warm germination rates were greater than 92% which is considered commercially acceptable."</p> <p>This conclusion is not acceptable. This study was not undertaken to test the seed lots on germination and to classify it whether the seed is suitable for commercial use or not. In this case, differences between the seeds should be observed and in case of statistically significant differences the test should be replicated and/or further questions and answers on the reasons of the differences should be undertaken.</p> <p>[Annex 28, Evaluation of germination and viability of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: Controlled environment test site. Dossier EFSA/GMO/NL/2015/127.]</p>	
Austria	Federal Ministry of Health	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	<p>The field trials were conducted during one year only. The number of sites (12) is adequate and the 4-fold replication is positive. Each block consisted of 4 rows with 2 main rows and 2 border rows.</p> <p>The measurement of 13 endpoints is rather small (c.f. Chapter 1.3.3.2).</p>	<p>For the comparative analysis, two independent sets of field trials were received: North America (2012) and North America (2015) (see spontaneous information received on the 30/5 and 6/6 2016), both sets were considered, although for different purposes. Compositional data were provided only for the field trials in 2012.</p>

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		<p>No distinction is made between specific leaf diseases; they are all characterised as "disease incidence". There is also no distinction made between pests, all are categorised as "insect damage".</p> <p>In the whole experiments, there were only small differences between treated, not treated GM maize and the conventional counterpart observed.</p> <p>It is very improbable, however, that there was no natural variation observed at site RG023NE7 (cf. FROM CBI: Annex 21 (Raw data file "PHI-2012-023_GMO_AGdata.xlsx")):</p> <ul style="list-style-type: none"> <li>• Time to Pollen Shed (accumulated heat units) - all 8 replicates (treated + not treated) have value "1356"</li> <li>• Time to Silking (accumulated heat units) - all 8 replicates (treated + not treated) have value "1306.5"</li> <li>• Seedling vigor - all 12 replicates (GM + conv. counterpart) have value "8"</li> <li>• Stalk lodging - all 12 replicates have value "0"</li> <li>• Root lodging - all 12 replicates have value "0"</li> <li>• Stay green - all 8 replicates (GM maize) have value "4"</li> <li>• Disease incidence - all 12 replicates have value "9"</li> <li>• Insect damage - all 12 replicates have value "9"</li> </ul> <p>The careless observations for site RG023NE7 make this field trial worthless. Furthermore, the "uniform" results wrongly indicate there are no differences between the tested maize varieties, and they have an influence on outcomes of the across-site analysis.</p> <p>The notifier is requested to provide a clarification regarding the field trial observations for site RG023NE7.</p> <p>[Annex 21, Agronomic characteristics of a maize line containing the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6: U.S. and Canada test sites (EU study format). Dossier EFSA/GMO/NL/2015/127.]</p>	<p>Agronomic-phenotypic data were measured in both sets, but they were assessed based only on data from the field trials in 2015, as those in 2012 did not include the measurement of yield components (yield and kernel weight) and therefore were considered incomplete.</p>
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Austria	Federal Ministry of Health	II.1.4 Toxicology	<p>The applicant does not present a whole plant feeding study with the stacked GM maize 1507xMON810xMIR162xNK603 with the exception of a broiler feeding study (FROM CBI: Annex 24) designed to evaluate nutritional equivalence but not toxicological effects. The assessment of toxic effects is mainly based on the risk assessment of the single events and reference is also made to the risk assessments of various sub-combinations (Scientific Information, p. 92). No specific data are presented to test for potential synergistic, additive or antagonistic effects. Apart from the stability of the inserts, however, EFSA recommends that the risk assessment of stacked event GMPs is focused on potential interactions between the traits in addition to the assessment of stability of the transgenic inserts in the stacked event (EFSA 2010). At the same time no agreed approach of testing for potential combinatory effects is available. However, scientific evidence exists that the current assumptions regarding the narrow specificity of Cry toxins or their mode of action have to be questioned (Hilbeck and Otto 2015). Therefore, we recommend comparing results from laboratory bioassays with the target organisms conducted with single toxins with those conducted with different combinations of the expressed toxins in order to check for indications of potential combinatory effects.</p> <p>1.4.2 Assessment of newly expressed proteins:  As regards the expression of proteins derived and modified from <i>Bacillus thuringiensis</i> (Bt), the Scientific Information (p. 29) and the notifier state, "Microbial preparations of Bt containing Cry proteins have been used safely as pesticide sprays for decades, and have been deemed to pose no toxic effects to mammals (Koch et al., 2015; US-EPA, 2001)."  According to the EPA database for registered pesticide products (PPLS) (as of March 31st, 2016), many Bt proteins expressed by GM plants are not registered to be used in pesticide sprays in the United States</p>	The GMO Panel takes note of the comment.
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		<p>(<a href="https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1">https://iaspub.epa.gov/apex/pesticides/f?p=PPLS:1</a>). Hence, the notifier should clarify whether Bt proteins as expressed by GM maize 1507xMON810xMIR162xNK603 have been used safely as pesticide sprays, in which countries, and for how long.</p> <p>The food safety of the newly expressed proteins, furthermore, is mainly based on acute feeding studies. The research programme "Food Safety in Europe (FOSIE): Risk Assessment of Chemicals in Food and Diet" which was supported by the European Commission, DG Research, considered acute feeding studies being "not particularly useful for hazard identification and risk assessment in relation to foods and food chemicals because human exposure tends to be much lower than that which causes acute toxicity and to continue over much longer periods " (Smith 2002).</p> <p>This is an opinion that is shared as well by EFSA (2011).</p> <p>Only the Vip3Aa20 protein has been tested via repeated-dose 28-day oral toxicity study in rats, and also for its potential for in vitro cytotoxicity in a study using human epithelial colorectal adenocarcinoma cell lines (cf. Additional Information of September 2010 submitted for Notification EFSA-GMO-DE-2010-82). With the exception of Vip3Aa20 protein, however, no mode of action tests in appropriate models reflecting mechanisms and processes in human cell systems were conducted. These tests are requested to be performed with Cry1F and the Cry1Ab to study their toxicological potential as well.</p> <p>One factor the risk potential to be rather minor is the slight assumable exposure to these proteins. Nevertheless, considerations on and evidence of the modes of actions of the single proteins - and also protein combinations - regarding effects on the human and animal organism are necessary to complete the toxicological risk assessment.</p> <p>4.5 Assessment of the whole food and/or feed derived</p>	
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		<p>from GM plants:  The compositional analysis revealed a high number of significant differences (&gt; 55% of the analytes evaluated) which leads to the conclusion that it is highly likely the genetic modification resulted in unintended effects. This fact should be given more attention regarding the toxicological assessment of the whole GM maize 1507xMON810xMIR162xNK603. Nevertheless, the notifier has not considered it necessary to carry out a 90-day toxicity study in rodents with the GM maize; and thus, no final evidence is possible with reference (even) to sub-chronic effects of the whole food and feed. Moreover, potential long-term (especially appropriate for foodstuffs), reproductive or developmental effects of the whole food and/or feed are not assessed by the notifier.</p> <p>[Annex 24, Nutritional equivalency study of the combined trait product DAS-Ø15Ø7-1xMON-ØØ81Ø-6xSYN-IR162-4xMON-ØØ6Ø3-6 - Poultry feeding study. Dossier EFSA/GMO/NL/2015/127.</p> <p>EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111.</p> <p>EFSA, 2011. Guidance of the GMO Panel for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5):2150: 1-37.</p> <p>Hilbeck A, Otto M, 2015. Specificity and combinatorial effects of Bacillus thuringiensis Cry toxins in the context of GMO environmental risk assessment. Frontiers in Environmental Science 3(71): 1-18.</p> <p>Smith M, 2002. Food Safety in Europe (FOSIE): risk assessment of chemicals in food and diet: overall introduction. Food Chem Toxicol 40(2-3): 141-144.]</p>	
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Austria	Federal Ministry of Health	II.6 Post-Market Environmental Monitoring Plan (PMEM)	<p><b>6.1 General:</b>  The notifier indicates that he is taking into account the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA 2006). However, a monitoring plan submitted in 2015 should rather be based on the current guidance document of EFSA for PMEM (EFSA 2011a).</p> <p>The proposed monitoring plan cannot be considered adequate for the following reasons:  The notifier does not specifically consider potential exposure of EU environments to GM maize 1507xMON810xMIR162xNK603 other than by unintended release of substantial volumes of GM maize 1507xMON810xMIR162xNK603 via losses during loading or unloading for processing into animal feed or human food products.</p> <p>Other exposure scenarios should be considered according to current EFSA guidance (EFSA 2011a), e.g. accidental spillage during transport, commingling with other maize grain lots and exposure via waste materials from processing or use. Since all exposure pathways should be taken into account in the monitoring plan appropriately, we consider the monitoring plan at hands to be insufficient to address the potential environmental effects of GM maize 1507xMON810xMIR162xNK603.</p> <p>The general recommendations by EFSA from the evaluation of previous monitoring of other GM crops (among others EFSA 2011b; EFSA 2012) should be considered by the notifier and appropriate suggestions, e.g. as regards the literature review, etc., should be implemented.</p> <p><b>6.3 Case-specific GM plant monitoring:</b>  The notifier does not present a plan for monitoring the exposure of the environment to GM maize 1507xMON810xMIR162xNK603 and materials derived from GM maize 1507xMON810xMIR162xNK603 using</p>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603. As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.</p>
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		<p>appropriate methods (i.e. standardised methodologies for sampling and identification of GM maize). Since the ERA presented for GM maize 1507xMON810xMIR162xNK603, in our opinion, is associated with uncertainties, Case Specific Monitoring (CSM) should be implemented to address the respective issues. Specifically, the extent of exposure of the environment to GM maize 1507xMON810xMIR162xNK603 and the fate of transgenic material in the environment should be addressed by CSM (c.f. Züghart et al. 2011). In the case that non-target organisms other than those tested by the notifier could be exposed to relevant amounts of transgenic materials derived from GM maize 1507xMON810xMIR162xNK603 either directly or indirectly, further testing should be conducted by the notifier under conditions of realistic levels of exposure as expected from commercial use of GM maize 1507xMON810xMIR162xNK603. Therefore, the notifier should submit a CSM plan appropriately addressing the above issues.</p> <p>6.4 General surveillance for unanticipated adverse effects:</p> <p>As noted in the general comments above all routes of exposure of the environment should be taken into account in GS, including exposure to (waste) materials from processing or use. The requirement that all potential routes of exposure should be addressed by the proposed monitoring is one of the pillars of the EU-approach to monitoring and included in the current EFSA guidance for PMEM (EFSA 2011a). The notifier states that he will monitor the occurrence of unanticipated adverse effects that have arisen from use of viable MON 1507xMON810xMIR162xNK603 (Scientific Information, Chapter 6.4.1, p. 142). Since exposure of the environment to transgenic material derived from GM maize 1507xMON810xMIR162xNK603 can also happen from non-viable GM maize 1507xMON810xMIR162xNK603 materials, the</p>	
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		<p>monitoring approach should not exclude exposure pathways originating from non-viable sources including processed GM maize 1507xMON810xMIR162xNK603. In addition, the description of the monitoring methodology does not exactly indicate which specific information will be gathered by General Surveillance. The notifier thus should describe in more detail the monitoring methodology and which data are gathered by GS and how.</p> <p>The notifier only states that the responsibilities for the General Surveillance of GM maize 1507xMON810xMIR162xNK603 are shared between the authorisation holder and third parties, such as operators involved in the import, handling and processing of viable GM maize 1507xMON810xMIR162xNK603 (e.g. traders, silo operators, processors). These operators, represented by trade associations and existing networks (e.g. COCERAL, UNISTOCK, FEDIOL), are obliged to report any potential unanticipated adverse effect to the authorisation holder.</p> <p>However, these organisations and companies are not specified in detail by the notifier. Thus, it remains unclear who will conduct the monitoring in practice. It is therefore not possible to evaluate the efficacy of the monitoring, which will be influenced by the availability, extent and composition of existing networks in EU Member States as well as their commitment as regards the monitoring goals.</p> <p>The notifier should therefore indicate which national organisations will be involved in each individual EU Member State and provide statements indicating their willingness to participate. It should be clarified which existing networks will be involved and to which degree they will be involved.</p> <p>Since the authorisation holder is responsible for ensuring that the monitoring plan included in the application is put in place and properly implemented, he should present appropriate information to justify his claim.</p>	
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		<p>Furthermore, the notifier has not selected other networks further down the food/feed production chain for General Surveillance. However, environmental effects of food/feed processing and the use of GM maize 1507xMON810xMIR162xNK603 in food or feed must be taken into account according to Regulation (EC) 1829/2003. Therefore, e.g. respective veterinary networks should be involved in particular for the surveillance of unanticipated effects on animal health. The methodology of the proposed General Surveillance is based on passively collecting information. However, in our view, a proactive approach for GS, including specific activities for monitoring of accidental spillage and the potential establishment of GM maize 1507xMON810xMIR162xNK603 in the environment, should be proposed and implemented by the notifier (see general remarks to this notification).</p> <p>The notifier states that the surveillance is based on HACCP principles without giving details on the specific approach. Thus, it is unclear how these principles match with the requirements of environmental monitoring of GM maize 1507xMON810xMIR162xNK603. The general reference to HACCP principles as included in the monitoring plan thus needs to be better specified by the notifier.</p> <p>In conclusion, the proposed monitoring plan is not considered appropriate to address relevant issues of PMEM of GM maize 1507xMON810xMIR162xNK603 and cannot be regarded as sufficiently elaborated for the monitoring of potential environmental exposure by GM maize 1507xMON810xMIR162xNK603.</p> <p>[EFSA, 2006. Guidance document of the Scientific Panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100.</p> <p>EFSA, 2011a. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal</p>	
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			<p>9(8):2316: 1-40.</p> <p>EFSA, 2011b. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. The EFSA Journal 9(10):2376: 1-66.</p> <p>EFSA, 2012. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. The EFSA Journal 10(4):2610: 1-35.</p> <p>Züghart W, Raps A, Wust-Saucy A-G, Dolezel M, Eckerstorfer M, 2011. Monitoring of genetically modified organisms. A policy paper representing the view of the National Environment Agencies in Austria and Switzerland and the Federal Agency for Nature Conservation in Germany. Umweltbundesamt Reports 305. Vienna: 1-56.]</p>	
Belgium	Biosafety Advisory Council	II.1.2.1 Information relating to the genetic modification	<p>Page 26 and Annex 7 (pages 9 and 13, and later): the toxin similarity search used as 'threshold of significance' an E value of <math>1 \times 10^{-5}</math>. Could the applicant justify why? Such threshold of significance is not adopted for some other expressed proteins within the same dossier (see e.g. Cry1F) and the rationale for such discrepancies should be given. This highlights the need for harmonisation in the reporting of similarity searches.</p>	<p>The applicant spontaneously submitted updated bioinformatic information (spontaneous information 17/12/2019 and 18/03/2020). The similarity search to identify similarity to predicted toxins was conducted with E-score cut-off value of <math>1 \times 10^{-5}</math> or a more conservative <math>1 \times 10^{-4}</math>. These cut-off values are considered appropriate to conduct similarity search to identify relevant hits in toxin databases (Pearson WR et al., 2000).</p> <p>Pearson WR (2000) Flexible sequence similarity searching with the FASTA3 program package. Methods in Molecular Biology 132: 185-219.</p>



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Belgium	Biosafety Advisory Council	II.1.2.2 Information relating to the genetically modified plant	Protein expression (table 11 page 52 of main dossier): for Vip3Aa20, a two-fold difference in the mean value is noticed when comparing the intended vs conventional herbicide treatments in the 4-event stack. No comment is made by the applicant. Is it possible that this difference is caused by the herbicide regime? Was this already observed with previously assessed subcombinations? The applicant should comment this difference in protein levels.	A variation in protein expression levels is not unexpected under different conditions. The data on Vip3Aa20 levels shows variation with or without treatment and the ranges overlap. Values were assessed by the EFSA GMO Panel and were not considered to pose a safety concern or indicate any interaction between the events in the stack.
France	DGCCRF	II.1.2 Molecular Characterisation	<p>II.1. Identification et caractérisation des dangers  II.1.2. Caractérisation moléculaire  II.1.2.4. Conclusions de la caractérisation moléculaire  Les éléments présentés dans le dossier relatifs à la caractérisation moléculaire des maïs génétiquement modifiés 1507 x MIR162 x MON810 x NK603 ne sont pas évocateurs d'un risque lié à l'utilisation de ces maïs en alimentation animale ou humaine. En ce qui concerne les sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603, seuls les hybrides NK603 x MON810 et TC1507 x NK603 disposent d'une caractérisation moléculaire, réalisée dans le cadre de leur demande d'autorisation de mise sur le marché</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1. Identification and characterisation of hazards  II.1.2. Molecular characterisation  II.1.2.4. Conclusions of the molecular characterisation  The evidence presented in the dossier, which relates to the molecular characterisation of the genetically modified maize line 1507 x MIR162 x MON810 x NK603, does not raise any particular issues associated with the use of this maize for human or animal consumption. For the sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, only the NK603 x MON810 x NK603 and TC1507 hybrid have undergone a molecular characterisation as part of an application for marketing authorisation</p>	The GMO Panel takes note of the comment.

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France	DGCCRF	<p>II.1.3.1 Choice of the conventional counterpart and additional comparators</p>	<p>II.1.3.1. Choix de l'équivalent non transgénique et des comparateurs supplémentaires</p> <p>L'évaluation comparative a été menée sur du maïs 1507 x MIR162 x MON810 x NK603 obtenu par croisement des maïs 1507 x MON810 (fonds génétique PHH9H) et MIR162 x NK603 (fonds génétique PHE4N). Ce maïs est comparé à juste titre avec une variété témoin non génétiquement modifiée dont le fonds génétique est PHH9H x PHE4N. Il est également comparé avec un total de 14 et 16 variétés commerciales conventionnelles de maïs pour, respectivement, l'analyse de composition et la caractérisation agronomique et phénotypique. Le pétitionnaire indique que ces variétés ont été choisies pour représenter la variabilité génétique, phénotypique, agronomique et de composition chimique des variétés conventionnelles de maïs. Il aurait été souhaitable d'avoir des informations sur leur représentativité vis-à-vis de l'ensemble des variétés cultivées dans le monde, et en particulier des variétés cultivées en Europe.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.1. Choice of the conventional counterpart and additional comparators.</p> <p>The comparative evaluation was carried out on the maize line MIR162 x 1507 x MON810 x NK603 by crossing them with MON810 x 1507 (PHH9H gene pool) and MIR162 x NK603 maize (PHE4N gene pool). This maize is justifiably compared with a non-GM control variety from the PHH9H x PHE4N gene pool. It is also compared to a total of 14 conventional and 16 commercial varieties of maize for, respectively, a compositional analysis and an agronomic and phenotypic characterisation. The applicant states that these varieties were selected to represent the genetic, phenotypic and agronomic variability, and the variability in chemical composition, of the conventional varieties of soya. It would have been desirable to have information about how representative they actually are</p>	<p>The EFSA GMO Panel thanks France for the summary.</p>
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			with respect to all of the varieties cultivated in the world, and in particular, varieties cultivated in Europe.	
France	DGCCRF	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	<p>II.1.3.2. Dispositif expérimental et analyse statistique des données issues des essais au champ pour l'analyse comparative</p> <p>Le maïs 1507 x MIR162 x MON810 x NK603 et la variété témoin ont été cultivés sur 12 sites aux USA et au Canada en 2012, de même que les variétés commerciales de référence (3 variétés par site). Le maïs génétiquement modifié a été cultivé sur chaque site avec 2 modalités : traité ou non avec du glyphosate et du glufosinate-ammonium (T et NT, respectivement). Chaque modalité (variété génétiquement modifiée T et NT, variété témoin et variétés commerciales) a été répétée quatre fois sur chaque site selon un plan d'expérience en blocs randomisés. Les caractéristiques de ce plan d'expérience respectent les recommandations du Panel GMO de l'EFSA (2011a).</p> <p>L'évaluation comparative repose sur des analyses de variance (ANOVA) réalisées en regroupant les résultats des 12 sites expérimentaux pour la caractérisation agronomique et phénotypique et en utilisant les résultats de 8 sites choisis au hasard parmi les 12 pour l'analyse de composition. Le maïs 1507 x MIR162 x MON810 x NK603 (T et NT) est comparé à la variété témoin par des tests de différence et aux variétés commerciales de référence par des tests d'équivalence. Pour un des paramètres de la caractérisation agronomique et phénotypique, il n'est pas possible de conclure, car l'absence de variabilité entre les variétés commerciales pour ce paramètre ne permet pas de calculer les limites d'équivalence.</p> <p>L'ANOVA est réalisée avec un modèle linéaire à effets mixtes incluant :</p> <ul style="list-style-type: none"> <li>- un effet fixe "génotype" (indiquant s'il s'agit du maïs 1507 x MIR162 x MON810 x NK603 (T ou NT), de la variété témoin ou des variétés commerciales) ;</li> <li>- des effets aléatoires : "site", "bloc dans le site" et</li> </ul>	The EFSA GMO Panel thanks France for the summary.

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		<p>"variété commerciale".</p> <p>L'erreur de type 1 retenue par le pétitionnaire est de 10 % pour les tests de différence et de 5 % pour les tests d'équivalence. Le modèle statistique utilisé, qui inclut un effet fixe "génotype" et un effet aléatoire "variété commerciale", correspond à celui proposé par le Panel GMO de l'EFSA (2011a).</p> <p>Les résultats des tests statistiques sont interprétés selon l'approche décrite par le Panel GMO de l'EFSA (2010), en classant les variables en 4 catégories selon les résultats du test d'équivalence et 7 types après combinaison avec les résultats des tests de différence.</p> <p>L'ensemble des modèles et méthodes (traitement des outliers, simulation study, etc.) sont pleinement décrits dans les annexes. Les données brutes sous format électronique et les programmes de calcul sont fournis.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.2. Experimental design and statistical analysis of data from field trials for comparative analysis. The maize line MON87705 x MON87708 x MON89788 and the A3525 variety were cultivated at twelve sites in Argentina during the 2013/2012 season, along with commercial varieties (3 varieties per site). The genetically modified maize was cultivated at each site following two different procedures: they were either treated or not treated with glyphosate and dicamba (respectively T and NT). Each procedure (for the genetically modified varieties T and NT, the control variety and commercial varieties) was repeated four times at each site in randomised blocks, in accordance with the experimental design. The characteristics of this experimental design comply with recommendations of the EFSA's GMO Panel (2011). The benchmarking is based on an analyses of variance (ANOVA) made by combining the results for agronomic and phenotypic characterisation from twelve experimental sites and the results of eight randomly</p>	
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		<p>selected sites, chosen from among the twelve, for compositional analysis. The maize line MON87427 x MON89034 x NK603 (T and NT) was compared with the control variety using difference tests and, with the commercial variety, using equivalence tests. For one of the parameters it was not possible to draw any conclusions, as the equivalence limits could not be established because of a lack of variability between the commercial varieties for this parameter.</p> <p>The ANOVA is carried out using a linear mixed effects model which includes:</p> <ul style="list-style-type: none"> <li>- a 'genotype' fixed effect (to find out if is from the maize line MON87427 x MON89034 x NK603 (T or NT), the control variety or the commercial varieties),</li> <li>- random effects: for each 'site', 'site block' and 'commercial variety'</li> </ul> <p>The type-1 error levels chosen by the applicant are 10% for the difference tests and 5% for the equivalence tests. The statistical model used, which includes a 'genotype' fixed effect and a 'commercial variety' random effect, corresponds to the one proposed by the EFSA's GMO Panel (2011).</p> <p>The results of the statistical tests are interpreted following the approach described by the EFSA's GMO Panel (2010). The variables were classified into four categories, according to the results of the equivalence test, and seven types after factoring in the results of the difference tests.</p> <p>All of the models and methods used (treatment of outliers, simulation studies, etc.) are fully described in the annexes. The raw data and calculation programmes are provided in electronic format.</p>	
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France	DGCCRF	II.1.3.3 Selection of material and compounds for analysis	<p>II.1.3.3. Sélection du matériel et des composés pour analyse</p> <p>Les composés analysés correspondent à ceux du document consensus de l'OCDE (2002), à l'exception de la vitamine C, du sélénium et du DIMBOA, auxquels le pétitionnaire a ajouté une vingtaine de composés. Bien que ces choix ne soient pas expliqués dans le dossier, les analyses réalisées sont jugées recevables.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.3. The selection of material and compounds for analysis.</p> <p>The compounds tested correspond to those in the OECD Consensus (2002), with the exception of vitamin C, selenium and DIMBOA, and the applicant added twenty compounds. Although these choices were not explained in the submission the analyses carried out were deemed to be acceptable.</p>	The EFSA GMO Panel thanks France for the summary.
France	DGCCRF	II.1.3.4 Comparative analysis of composition	<p>II.1.3.4. Analyse comparative de la composition</p> <p>Les mesures de 71 composés (62 dans les grains et 9 dans le fourrage) parmi les 92 analysés sont utilisables pour les analyses statistiques. En effet, les résultats relatifs à 8 composés sont inférieurs à la limite de quantification (LOQ) de la méthode de mesure dans toutes les modalités (variété génétiquement modifiée T et NT, variété témoin et variétés commerciales de référence). Par ailleurs, 13 composés sont exclus de l'analyse car plus de 50 % des valeurs mesurées sont inférieures à la LOQ. Des tests exacts de Fisher conduits sur ces 13 paramètres montrent que leur nombre est équivalent dans les différents groupes. Par ailleurs, une étude complète par simulation sur le nombre de différences significatives attendues est fournie.</p> <p>L'analyse combinée de l'ensemble des sites d'expérimentation montre que la composition des grains et du fourrage du maïs 1507 x MIR162 x MON810 x NK603, T et NT, est équivalente à celle des</p>	The EFSA GMO Panel thanks France for the summary.

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			<p>variétés commerciales (tous les composés sont classés en catégorie I ou II).</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.4. Comparative analysis of the composition  The measurements of 71 compounds (65 in grain and 9 in feed), out of the total of 92 analysed, are usable for the statistical analyses. In fact, the results for eight compounds are below the limit of quantification (LoQ) for the measurement method used for all samples (genetically modified variety T and NT, the control variety and commercial reference varieties). In addition, 13 compounds have been excluded from the analysis because more than 50% of the measured values were below the LoQ. Fisher's exact tests, carried out on these 13 parameters, show that their values are equivalent in all groups. In addition, the results of a comprehensive simulation study on the expected number of significant differences have been provided.</p> <p>The combined analysis from all the experimental sites shows that the composition of grain and forage from the maize line MIR162 x 1507 x MON810 x NK603, T and NT, is equivalent to that of commercial varieties (all compounds are classified class I or II).</p>	
France	DGCCRF	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	<p>II.1.3.5. Analyse comparative des caractéristiques agronomiques et phénotypiques  Les caractéristiques agronomiques et phénotypiques ont été évaluées sur 20 paramètres. Les résultats obtenus pour 14 de ces paramètres sont utilisables pour les analyses statistiques : l'un des paramètres ne peut pas être catégorisé (Cf. II.1.3.2.) et pour 5 paramètres, plus de 50 % des résultats prennent la même valeur. Le maïs 1507 x MIR162 x MON810 x NK603, T ou NT, apparaît équivalent aux variétés commerciales de référence sur le plan agronomique et phénotypique, à l'exception de la tolérance aux herbicides glufosinate-ammonium et glyphosate.</p>	The EFSA GMO Panel thanks France for the summary and reminds that additional field trials were spontaneously submitted by the applicant. See section 3.4.2 of the scientific opinion.

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			<p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.5. The comparative analysis of agronomic and phenotypic characteristics  The agronomic and phenotypic characteristics were assessed with reference to 20 parameters. The results from 14 of these parameters can be used for statistical analyses: one of the parameters cannot be categorized (Cf. II.1.3.2.) and for five parameters over 50% of the results have the same value. The maize line MON87705 x MON87708 x MON89788, T or NT, appears to be equivalent to the commercial varieties in its agronomic and phenotypic character, with the exception of its tolerance to the herbicides glyphosate and dicamba.</p>	
France	DGCCRF	II.1.3.6 Effects of processing	<p>II.1.3.6. Effets de la transformation  Le pétitionnaire affirme que les produits issus du maïs 1507 x MIR162 x MON810 x NK603 ne devraient pas être différents de ceux issus de maïs conventionnels et ne présente pas d'analyse des produits transformés.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.6. The effects of processing  The applicant argues that the products derived from the maize line MON87705 x MON87708 x MON89788 should be no different from those derived from conventional maize, and does not present an analysis of the processed products.</p>	The EFSA GMO Panel thanks France for the summary.
France	DGCCRF	II.1.3.7 Conclusion	<p>II.1.3.7. Conclusions de l'évaluation comparative  L'analyse de composition réalisée sur les grains et le fourrage, ainsi que la caractérisation agronomique et phénotypique du maïs 1507 x MIR162 x MON810 x NK603, traité ou non avec les herbicides glufosinate-ammonium et glyphosate, montrent que ce maïs est équivalent aux variétés de maïs conventionnelles. Aucune analyse n'a été réalisée sur les produits issus du maïs 1507 x MIR162 x MON810 x NK603.</p> <p>En ce qui concerne les sous-combinaisons contenant deux ou trois des événements TC1507, MIR162,</p>	The EFSA GMO Panel thanks France for the summary.



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			<p>MON810 et NK603, seuls les hybrides NK603 x MON810 et TC1507 x NK603 disposent d'une évaluation comparative, réalisée dans le cadre de leur demande d'autorisation de mise sur le marché. Les analyses ont montré que ces deux hybrides étaient équivalents aux variétés de maïs conventionnelles.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.3.7. Conclusions of the comparative assessment  A compositional analysis carried out on the seeds and the fodder, as well as the agronomic and phenotypic characterisation of the maize line 1507 x MIR162 x MON810 x NK603, treated or not treated with the herbicides glyphosate and dicamba, show that this maize is equivalent to conventional maize varieties. No analysis was carried out on the products derived from the maize line MON87427 x MON89034 x NK603.</p> <p>For the sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, only the NK603 x MON810 x NK603 and TC1507 hybrid have undergone a comparative evaluation, as part of their application for marketing authorisation. The analyses showed that these two hybrids were equivalent to conventional maize varieties.</p>	
France	DGCCRF	II.1.4.1 Testing of newly expressed proteins	<p>II.1.4.1. Analyse des protéines nouvellement exprimées  Le pétitionnaire renvoie aux évaluations antérieures des protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS réalisées par l'EFSA et à leurs conclusions favorables. Par ailleurs, l'analyse bioinformatique actualisée des séquences de ces protéines ne met pas en évidence d'homologies avec des toxines ou des allergènes connus. Enfin, chacune de ces protéines a fait l'objet d'un test de toxicité aiguë orale sur rongeur lors des demandes d'autorisation de mise sur le marché des quatre maïs parentaux. Aucun effet toxique n'a été observé aux doses testées (576, 5000, 1250, 3030, 4000 et 572 mg/kg de poids corporel pour Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS,</p>	

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			<p>respectivement). La protéine CP4 EPSPS L214P n'a pas fait l'objet d'un test de toxicité aiguë orale sur rongeur, mais l'absence de cette étude est jugée acceptable par le GT « Biotechnologie » dans ce cas.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.4.1. Testing of newly expressed proteins  The applicant refers to previous evaluations of the proteins Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS carried out by the EFSA, and their favourable conclusions. The updated bioinformatic analysis of the sequences of these proteins does not show any evidence of homology with known toxins or allergens. Each of these proteins has been the subject of an acute oral toxicity test in rodents during the application for marketing authorisation for four parent maize varieties. No toxic effects were observed at the doses tested (576, 5000, 1250, 3030, 4000 and 572 mg/kg body weight for Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS, respectively). The CP4 EPSPS L214P protein has not been the subject of an acute oral toxicity test in rodents, but the fact that there has been no such test is acceptable to the 'Biotechnology' WG, in this instance.</p>	The EFSA GMO Panel thanks France for the summary.
France	DGCCRF	II.1.4.2 Testing of new constituents other than proteins	<p>II.1.4.2. Analyse des nouveaux constituants autres que les protéines  Le pétitionnaire ne fournit pas d'information sur la présence éventuelle de nouveaux constituants.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.4.2. Analysis of new constituents other than proteins  The applicant does not supply any information on the possible presence of new constituents.</p>	The GMO Panel takes note of the comment.
France	DGCCRF	II.1.4.3 Information on natural food and feed constituents	<p>II.1.4.3. Informations sur les constituants naturels de la denrée alimentaire ou de l'aliment pour animaux  Aucune analyse n'a été réalisée sur des denrées alimentaires ou des aliments pour animaux dérivés du maïs 1507 x MIR162 x MON810 x NK603.</p>	The GMO Panel takes note of the comment.

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			<p><b>ENGLISH TRANSLATION</b></p> <p>II.1.4.3. Information on the natural constituents of the foodstuff or animal feed  No analysis has been carried out on foodstuffs or animal feeds derived from MON87705 x MON87708 x MON89788 maize.</p>	
France	DGCCRF	II.1.4.4 Testing of the whole genetically modified food or feed	<p>II.1.4.4. Analyse de l'aliment (denrée alimentaire ou aliment pour animaux) génétiquement modifié entier  Aucune étude de toxicité sub-chronique de 90 jours sur rongeur n'a été réalisée avec le maïs 1507 x MIR162 x MON810 x NK603.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.4.4. Analysis of the whole range of genetically modified food (foodstuff or animal feed)  The maize line MON87705 x MON87708 x MON89788 has not been the subject of a 90-day sub-chronic oral toxicity test in rodents.</p>	The GMO Panel takes note of the comment.
France	DGCCRF	II.1.4.5 Conclusion of the toxicological assessment	<p>II.1.4.5. Conclusions de l'évaluation toxicologique  Les études de toxicité sub-chronique de 90 jours chez le rat réalisées avec chacun des maïs parentaux n'a pas permis d'identifier un risque sanitaire lié à la consommation de grains et produits dérivés de ces maïs. Par ailleurs, sur la base des éléments présentés dans le dossier, le maïs 1507 x MIR162 x MON810 x NK603 apparaît équivalent aux variétés de maïs conventionnelles. Enfin, le pétitionnaire présente un argumentaire détaillé et conforme aux recommandations de l'EFSA (2011a) en matière d'interactions potentielles entre les événements TC1507, MIR162, MON810 et NK603 au sein du maïs 1507 x MIR162 x MON810 x NK603. Dans ces conditions, l'absence d'étude de toxicité sub-chronique de 90 jours sur rongeur pour ce maïs est acceptable.</p> <p>En ce qui concerne les sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603, aucune d'entre elles n'a fait l'objet</p>	

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			<p>d'une étude de toxicité sub-chronique de 90 jours sur rongeur.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.4.5. Conclusions of the toxicological assessment  The 90-day sub-chronic oral toxicity studies in rodents carried out on each of the parent maizes has not identified any health risks associated with the consumption of grains and products derived from this maize. Furthermore, based on the evidence presented in the dossier, the maize line 1507 x MIR162 x MON810 x NK603 appears to be equivalent to conventional maize varieties. Finally, the applicant presents a detailed argument compliant with the EFSA's recommendations (2011a) on the potential interactions between the TC1507, MIR162, MON810 and NK603 events in the maize line 1507 x MIR162 x MON810 x NK603. In view of this, the fact that this maize has not been the subject of a 90-day sub-chronic toxicity study on rodents is acceptable.</p> <p>For the sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, none of them has been the subject of a 90-day sub-chronic toxicity test in rodents</p>	<p>The GMO Panel takes note of the comment and thanks for the summary.</p>
France	DGCCRF	II.1.5.1 Assessment of allergenicity of the newly expressed protein	<p>II.1.5.1. Évaluation de l'allergénicité de la (des) protéine(s) nouvellement exprimée(s)  Le pétitionnaire suit les recommandations du Panel GMO de l'EFSA (2011a) et fonde l'évaluation de l'allergénicité des protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS exprimées dans le maïs 1507 x MIR162 x MON810 x NK603 sur quatre critères :</p> <p>1) absence d'allergénicité connue des organismes sources (Bacillus thuringiensis, Streptomyces viridochromogenes, Escherichia coli et Agrobacterium sp.) ;</p> <p>2) absence d'identités globale et locale de séquence des six protéines, Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS, avec des allergènes connus ;</p>	<p>The GMO Panel takes note of the comments and thanks for the summary.</p>

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		<p>3) faible résistance des six protéines à la protéolyse digestive ;</p> <p>4) faible teneur en protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS des grains du maïs 1507 x MIR162 x MON810 x NK603.</p> <p>Les analyses bioinformatiques ne montrent aucune homologie de séquence entre les six protéines nouvellement exprimées et les adjuvants classiques comme les toxines. Par ailleurs, les faibles teneurs de ces protéines dans le maïs 1507 x MIR162 x MON810 x NK603 et leur sensibilité aux protéases digestives sont a priori incompatibles avec un éventuel effet adjuvant significatif dans le cadre d'un apport alimentaire modéré en maïs génétiquement modifié. Plusieurs publications récentes ayant mentionné le caractère adjuvant des protéines Cry, le pétitionnaire analyse de façon convaincante les résultats de ces publications qui montrent que le caractère adjuvant :</p> <ul style="list-style-type: none"> <li>- n'est observé chez l'animal qu'à une dose élevée ;</li> <li>- ne fonctionne pas avec tous les antigènes ;</li> <li>- n'est observé qu'après administration des protéines Cry par voie intra-gastrique ;</li> <li>- est observé lorsqu'un anti-acide qui bloque la protéolyse pepsique est administré en même temps que les protéines Cry.</li> </ul> <p>Le pétitionnaire estime, à juste titre, qu'il s'agit là de conditions d'utilisation des protéines Cry non comparables à celles liées à la consommation du maïs 1507 x MIR162 x MON810 x NK603 et que le caractère adjuvant des protéines Cry associées à des PGM n'a jamais été démontré.</p> <p>Une analyse comparée de la structure des protéines CP4 EPSPS et CP4 EPSPS L214P réalisée par le GT « Biotechnologie » montre que la protéine CP4 EPSPS L214P ne diffère pas de la protéine CP4 EPSPS en termes d'allergénicité potentielle.</p> <p>L'ensemble de ces résultats suggère que les sept</p>	
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		<p>protéines nouvellement exprimées dans le maïs 1507 x MIR162 x MON810 x NK603 ne sont pas des allergènes potentiels et qu'il est peu vraisemblable que l'expression de ces protéines dans ce maïs ou dans les dix sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603 puisse entraîner une réaction allergique.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.5.1. Assessment of allergenicity of the newly expressed proteins.</p> <p>The applicant follows the recommendations of the EFSA's GMO Panel (2011a) and bases their evaluation of the allergenicity of proteins Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS expressed in the maize line MIR162 x 1507 x MON810 x NK603 on four criteria:</p> <ol style="list-style-type: none"> <li>1) no known allergenicity of the source organisms (<i>Bacillus thuringiensis</i>, <i>Streptomyces viridochromogenes</i>, <i>Escherichia coli</i> and <i>Agrobacterium</i> sp.)</li> <li>2) the absence of any global or local sequence identity for the six proteins, Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS with known allergens</li> <li>3) the low proteolytic resistance of the three proteins</li> <li>4) a low level of the proteins Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS in grains from the maize line 1507 x MIR162 x MON810 x NK603.</li> </ol> <p>The bioinformatics analyses show no sequence homology between the six newly expressed proteins and the classical adjuvants such as toxins or lectins. In addition, the low level of these proteins in the maize line MON87427 x MON89034 x NK603 and their sensitivity to digestive proteases are, by definition, incompatible with any significant adjuvant effect following moderate dietary intake of genetically modified maize. Several recent publications have mentioned the adjuvant character of Cry proteins. The</p>	
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		<p>applicant's analysis have confirmed the results of these publications showing that the adjuvant effect:</p> <ul style="list-style-type: none"> <li>- is observed only in animals, at high doses</li> <li>- does not appear with all antigens</li> <li>- is observed only after the intragastric administration of Cry proteins</li> <li>- the adjuvant properties are observed when an antacid, which blocks pepsin proteolysis, is administered concomitantly with Cry proteins.</li> </ul> <p>The applicant believes, correctly, that this a result of protein usage conditions for Cry that are not comparable with those associated with the consumption of the maize line MIR162 x 1507 x MON810 x NK603 and the adjuvant character of Cry proteins associated with PGM enzymes has never been demonstrated.</p> <p>A comparative analysis of the structure of the proteins CP4 EPSPS and CP4 EPSPS L214P, carried out by the 'Biotechnology' WG, shows that the CP4 EPSPS L214P protein is no different from the CP4 EPSPS protein in terms of its potential allergenicity.</p> <p>All these results suggest that the seven newly expressed proteins in the maize line MIR162 x 1507 x MON810 x NK603 are not potential allergens and it is unlikely that the expression of these proteins in maize, or within the ten sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, would cause allergic reactions.</p>	
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France	DGCCRF	<p>II.1.5.2 Assessment of allergenicity of the whole genetically modified plant</p>	<p>II.1.5.2. Évaluation de l'allergénicité de la plante génétiquement modifiée entière</p> <p>Le pétitionnaire rappelle, à juste titre, que le maïs n'est pas considéré comme un allergène alimentaire majeur. Il ne figure pas dans la liste des allergènes dont l'étiquetage est obligatoire. En France, les statistiques du Réseau d'Allergo-Vigilance (RAV), qui recense les cas d'allergie alimentaire graves (chocs anaphylactiques), ne mentionnent pas le maïs dans la liste des 10 premiers allergènes dangereux (qui représentent 60 % des urgences allergologiques).</p> <p>Par ailleurs, aucune des informations disponibles au sujet du maïs 1507 x MIR162 x MON810 x NK603 ne laisse supposer que ce maïs puisse développer une allergénicité différente de celle des variétés de maïs conventionnelles. Le risque allergénique de ce maïs est faible et a priori équivalent à celui des variétés de maïs conventionnelles. Pour que ces conclusions puissent s'appliquer aux dix sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603, il est nécessaire de disposer des teneurs des protéines nouvellement exprimées dans ces maïs et de les comparer à celles des maïs parentaux, ce qui donnerait une indication des éventuelles interactions entre les événements dans les sous-combinaisons.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.5.2. Allergenicity assessment of the whole genetically modified plant</p> <p>The applicant correctly states that maize is not considered to be a major food allergen. It does not appear in the list of allergens requiring mandatory labelling. In France, statistics from the Allergy Vigilance network (RAV), which lists cases of serious food allergies (anaphylactic shock), makes no mention of maize on the list of the 10 leading hazardous allergens (which account for 60% of allergy emergencies).</p>	<p>The GMO Panel takes note of the comments.</p> <p>In relation to the question on interactions between events in these sub-combinations.</p> <p>Please see Section on the risk assessment of subcombinations detailed as follows: "Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining subcombinations included in the scope of application EFSA-GMO-NL-2015-127, no experimental data have been provided. For these subcombinations, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed subcombinations and the four-event stack maize".</p>
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			<p>Furthermore, none of the information available about maize MON87427 x MON89034 x NK603 suggests that this maize could develop a different allergenicity to that of conventional varieties of maize. The allergenic risk for this maize is low and, in principle, equivalent to that of conventional maize varieties. To verify that these conclusions also apply to the ten sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, it is necessary to compare the contents of the newly expressed proteins in these maize lines with those of the parent maize, which would give an indication of the possible interactions between events in these sub-combinations.</p>	
France	DGCCRF	II.1.5.3 Conclusion of the allergenicity assessment	<p>II.1.5.3. Conclusions de l'évaluation de l'allergénicité Sur la base des données et des commentaires fournis par le pétitionnaire :</p> <ul style="list-style-type: none"> <li>- le potentiel allergénique des protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab, CP4 EPSPS et CP4 EPSPS L214P exprimées dans le maïs 1507 x MIR162 x MON810 x NK603 peut être considéré comme négligeable ;</li> <li>- ces protéines ne possèdent apparemment aucune propriété adjuvante ;</li> <li>- l'allergénicité du maïs 1507 x MIR162 x MON810 x NK603 reste vraisemblablement identique à celle d'un maïs conventionnel ;</li> <li>- pour que ces conclusions puissent s'appliquer aux dix sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603, il est nécessaire de disposer des teneurs des sept protéines nouvellement exprimées dans ces maïs.</li> </ul> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.5.3. Conclusions of the allergenicity assessment. Based on the data and comments supplied by the applicant:</p> <ul style="list-style-type: none"> <li>- the allergenic potential of the proteins Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab, CP4 EPSPS and CP4 EPSPS</li> </ul>	<p>The GMO Panel takes note of the comments. It is unclear however, what is meant in the last comment on the ten sub-combinations. For more information, please see Section on the risk assessment of subcombinations detailed as follows: "Since no new safety concerns were identified for the previously assessed subcombinations, the GMO Panel considers that its previous conclusions on these maize subcombinations remain valid. For the remaining subcombinations included in the scope of application EFSA-GMO-NL-2015-127, no experimental data have been provided. For these subcombinations, the GMO Panel assessed the possibility of interactions between the events and concluded that these combinations would not raise safety concerns. These subcombinations are therefore expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed subcombinations and the four-event stack maize".</p>

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			<p>L214P expressed in the maize line MIR162 x 1507 x MON810 x NK603 can be considered negligible</p> <ul style="list-style-type: none"> <li>- these proteins apparently do not have any adjunctive properties.</li> <li>- the allergenicity of the maize line MON87427 x MON89034 x NK603 is virtually identical to that of conventional maize</li> <li>- in order that these conclusions can also be applied to the ten sub-combinations containing two or three of the TC1507, MIR162, MON810 and NK603 events, it is necessary to rank the level of these seven newly expressed proteins in the maize.</li> </ul>	
France	DGCCRF	II.1.6 Nutritional assessment	<p>II.1.6. Evaluation nutritionnelle</p> <p>Une étude d'alimentarité a été réalisée sur 720 poulets Ross 708 (360 mâles et 360 femelles) nourris pendant 42 jours avec trois régimes successifs (démarrage, croissance et finition) contenant 61,5 %, 66 % et 72,9 % de maïs, respectivement. Le maïs 1507 x MIR162 x MON810 x NK603 (fonds génétique PH581 x SYNTAX5707), NT et T, a été comparé avec une variété témoin non génétiquement modifiée de même fonds génétique et avec 3 variétés commerciales conventionnelles. Aucun effet significatif n'est observé. Par conséquent, pour le poulet de type standard en croissance, le maïs 1507 x MIR162 x MON810 x NK603 a les mêmes qualités nutritionnelles que le maïs témoin et les variétés de maïs conventionnelles testées dans cette étude.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.1.6. Nutritional assessment</p> <p>A food compatibility study was carried out on 720 Ross 708 chickens (360 males and 360 females) fed for 42 days following three successive diets (start-up, growth and finishing) containing 61.5 %, 66 % and 72.9 % of maize, respectively. The maize line 1507 x MIR162 x MON810 x NK603 (PH581 gene pool x SYNTAX5707), NT &amp; T, has been compared with a non-GM control variety from the same gene pool and with three</p>	The GMO Panel takes note of the comment.

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			conventional commercial varieties. No significant effect was observed. Therefore, for the standard type of growing chicken, the maize line MIR162 x 1507 x NK603 x MON810 has the same nutritional qualities as the control maize and conventional maize varieties tested in this study.	
France	DGCCRF	II.2 Exposure assessment — anticipated intake or extent of use	<p>II.2 Évaluation de l'exposition - Prévision de la quantité consommée ou de l'étendue de l'utilisation  L'estimation de la consommation de maïs chez l'animal est basée sur les données de l'OCDE (2009) . Dans un scénario du "pire des cas", la consommation la plus élevée concerne la protéine Vip3Aa20 (6,33 mg/kg p.c./jour chez la vache laitière).</p> <p>L'évaluation de l'exposition humaine aux protéines exprimées dans le maïs 1507 x MIR162 x MON810 x NK603 est fondée sur l'utilisation de l'EFSA Comprehensive European Food Consumption Database . Dans un scénario du "pire des cas", les expositions aiguë et chronique maximales concernent la protéine Vip3Aa20 (respectivement 0,27 et 0,016 mg/kg p.c./jour pour la catégorie "Other Children" (enfants de 3 à 9 ans) en Suède et au Danemark).</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.2 Exposure assessment - Anticipated intake/extent of use.  The estimation of maize consumption in animals is based on OECD data (2009). In a 'worst case' scenario, the Vip3Aa20 protein had the highest level (6.33 mg/kg body weight x day in dairy cows).</p> <p>The assessment of human exposure to proteins expressed in the MIR162 x 1507 x MON810 x NK603 maize line is based on the EFSA Comprehensive European Food Consumption Database In a 'worst case' scenario, the maximum acute and chronic levels were also for the Vip3Aa20 protein (0.27 and 0.016</p>	The GMO Panel takes note of the comment.

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			mg/kg bw x day respectively for the 'Other Children' category (children between 3 and 9 years old) in Sweden and Denmark).	
France	DGCCRF	II.3 Risk characterisation	<p>II.3 Caractérisation des risques</p> <p>En l'absence d'études de toxicité et d'alimentarité réalisées sur des animaux de rente (vaches laitières, poulets et porcs), le risque ne peut pas être caractérisé pour ces animaux.</p> <p>Chez l'Homme, le pétitionnaire présente un calcul des marges de sécurité fondé sur les résultats d'études de toxicité aiguë orale sur la souris des protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab et CP4 EPSPS. Le GT « Biotechnologie » considère que cette démarche n'est pas adaptée, car elle ne permet pas d'estimer le risque associé à une consommation répétée de produits issus du maïs 1507 x MIR162 x MON810 x NK603. Il aurait été plus pertinent de calculer une marge de sécurité à partir des NOAEL pouvant être déduites des études de toxicité sub-chronique de 90 jours plutôt que de celles déduites des études de toxicité aiguë. Néanmoins, compte tenu de la faible teneur en protéines Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab, CP4 EPSPS et CP4 EPSPS L214P dans les grains et de la faible résistance à la protéolyse digestive de ces protéines, les risques apparaissent négligeables.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.3 Risk characterisation</p> <p>In the absence of any toxicity and alimentarity studies on farm animals (cows, chickens and pigs), the risk cannot be characterised for these animals.</p> <p>In humans, the applicant has submitted a calculation of the safety margins based on the results of acute oral toxicity studies in mice for the proteins Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS. The 'Biotechnology' Working Group considers that this</p>	<p>The GMO Panel takes note of the comment and thanks for the summary.</p> <p>In the EFSA scientific opinion, risk characterization was not conducted when assessing and concluding on the safety of Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab and CP4 EPSPS proteins in humans. The GMO Panel thanks France for the comment.</p>

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			<p>approach is not suitable, since it does not make it possible to estimate the risk associated with the regular consumption of products derived from the maize line 1507 x MIR162 x MON810 x NK603. It would have been more appropriate to calculate a margin of safety from the NOAEL (no observable adverse-effect level) derived from the 90 day sub-chronic toxicity studies instead of those from acute toxicity studies. However, in view of the low levels of Cry1F, PAT, Vip3Aa20, PMI, Cry1Ab, CP4 EPSPS and CP4 EPSPS L214P proteins in the grain and the low resistance of this protein to digestive proteolysis, the risks appear to be negligible.</p>	
France	DGCCRF	II.4 Post-market monitoring on the genetically modified food or feed	<p>II.4 Surveillance de la denrée alimentaire ou de l'aliment pour animaux génétiquement modifié(e) consécutive à sa mise sur le marché  Le pétitionnaire n'a pas proposé de plan de surveillance de la denrée alimentaire ou de l'aliment pour animaux génétiquement modifié(e) consécutive à sa mise sur le marché.</p> <p><b>ENGLISH TRANSLATION</b></p> <p>II.4 Monitoring of the genetically modified food or animal feed after being placed on the market.  The applicant has not proposed a plan for monitoring the genetically modified food or animal feed after they become commercially available.</p>	As reflected in section 3.6.1., the GMO Panel concluded that the four-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the non-GM reference varieties tested. Therefore, post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary
France	DGCCRF	Part II – Scientific information	<p>Conclusions du Groupe de travail « Biotechnologie » ANSES  Les maïs 1507 x MIR162 x MON810 x NK603, objets de la présente saisine, sont issus de croisements conventionnels entre les maïs TC1507, MIR162, MON810 et NK603. Les informations moléculaires présentées dans le dossier permettent de caractériser ces maïs et ne sont pas évocatrices d'un risque pour la santé humaine et animale. Sur la base des éléments fournis par le pétitionnaire, le potentiel allergénique de ces maïs et de leurs produits dérivés paraît négligeable.</p>	

		<p>L'analyse de composition réalisée sur les grains et le fourrage, ainsi que la caractérisation agronomique et phénotypique des maïs 1507 x MIR162 x MON810 x NK603, traités ou non avec les herbicides glufosinate-ammonium et glyphosate, montrent que ces maïs sont équivalents aux variétés de maïs conventionnelles. Le pétitionnaire présente un argumentaire détaillé et conforme aux recommandations de l'EFSA (2011a) en matière d'interactions potentielles entre les événements TC1507, MIR162, MON810 et NK603 au sein du maïs 1507 x MIR162 x MON810 x NK603. Dans ces conditions, l'absence d'étude de toxicité sub-chronique de 90 jours sur rongeur pour ce maïs est acceptable. Enfin, l'étude d'alimentarité sur poulets montre que les maïs 1507 x MIR162 x MON810 x NK603 ont les mêmes qualités nutritionnelles que le maïs témoin et les variétés de maïs conventionnelles testées dans cette étude.</p> <p>L'ensemble de ces éléments ne permet pas d'identifier un risque sanitaire lié à l'utilisation de grains et produits dérivés des maïs 1507 x MIR162 x MON810 x NK603 en alimentation animale ou humaine.</p> <p>En ce qui concerne les dix sous-combinaisons contenant deux ou trois des événements TC1507, MIR162, MON810 et NK603, les hybrides NK603 x MON810 et TC1507 x NK603 ont fait l'objet d'une évaluation dans le cadre d'une demande d'autorisation de mise sur le marché. L'Agence a conclu que la consommation de ces maïs et de leurs dérivés présente le même niveau de sécurité sanitaire pour l'Homme et l'animal que la consommation de maïs non génétiquement modifiés et de leurs dérivés (Afssa, 2004f, 2005a, b et c). Pour les huit autres sous-combinaisons, le GT « Biotechnologie » considère qu'il ne dispose pas de suffisamment d'éléments pour statuer.</p> <p><b>ENGLISH TRANSLATION</b></p>	<p>The GMO Panel takes note of the comment.</p>
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		<p>Conclusions of the ANSES 'Biotechnology' Working Group</p> <p>The maize line 1507 x MIR162 x MON810 x NK603, the subject of this referral, is a product of the traditional crossing with TC1507, MIR162, MON810 and NK603 maize line. The molecular data presented in the dossier has been used to characterise this maize and does not suggest any risk to human or animal health. On the basis of the information provided in the dossier, the allergenic potential of products derived from this maize appears to be negligible.</p> <p>A compositional analysis carried out on the seeds and the fodder, as well as the agronomic and phenotypic characterisation of the maize line 1507 x MIR162 x MON810 x NK603, treated or not treated with the herbicides glyphosate and dicamba, show that this maize is equivalent to the conventional maize varieties. The applicant presents a detailed argument in line with EFSA's recommendations (2011a) on the potential interactions between the TC1507, MIR162, MON810 and NK603 events in the 1507 x MIR162 x MON810 x NK603 maize line. In view of this, the fact that this maize has not been the subject of a 90-day sub-chronic toxicity study on rodents is acceptable. Finally, the food compatibility study on chickens shows that 1507 x MIR162 x MON810 x NK603 have the same nutritional qualities as the control maize and the conventional maize varieties tested in this study. None of these elements identified a health risk related to the use of the grain and products of the 1507 x MIR162 x MON810 x NK603 maize line in food or feed.</p> <p>The ten sub-combinations containing two or three of the TC1507 events, and the MIR162, MON810 and NK603, MON810 x NK603 and TC1507 x NK603 hybrids have been assessed as part of an application for market authorization. For the maize line MIR162, MON87427 and NK603, the Agency concluded that the consumption of this maize and their derivatives</p>	
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			presents the same level of risk to health and safety for human beings and animals as the consumption of non-genetically modified maize and their derivatives. For the other eight sub-combinations, the 'Biotechnology' Working Group considers that it does not have sufficient data to make a decision.	
Germany	BfN	II.1 Hazard identification and characterisation	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The Federal Agency for Nature Conservation (BfN) considers that further information is required before the risk assessment of EFSA/GMO/NL/2015/127 can be finalised. In particular the impact of the genetic modification on plant metabolism (in particular stress response pathways) in 1507xMON810xMIR162xNK603 maize should be further investigated. Information provided on composition demonstrated major differences between 1507xMON810xMIR162xNK603 maize and the non-modified counterpart and a shift in metabolic equilibrium close to, or even beyond, the border of reference varieties. All observed alterations within different classes of substances correspond to stress response pathways and indicate a systemic upregulation of stress response in 1507xMON810xMIR162xNK603 maize (see II.1.3.4). Hence we reject the conclusion on substantial equivalence between 1507xMON810xMIR162xNK603 maize and the non-modified counterpart.</p> <p>Novel proteins synthesized by 1507xMON810xMIR162xNK603 maize may enter the environment mainly via loss and spillage or as waste products during processing and as part of the food/feed chain. The applicant failed to assess major exposure pathways and is required to analyze fate of Bt proteins within feed or waste products derived from 1507xMON810xMIR162xNK603 maize and its implications for the receiving environments. Furthermore, studies on the effect of the GM maize on human and animal health need to be improved and supplemented (see comments under A.4.).</p>	FF



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			<p>The applicant's proposal for an environmental monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC) and therefore should be amended before consent can be given. In addition, the uncertainties resulting from the above-mentioned differences between 1507xMON810xMIR162xNK603 maize and the non-modified counterpart should be reflected in the environmental monitoring plan.</p>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603. As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.</p>
Germany	BfN	II.1.3.4 Comparative analysis of composition	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>Results of the compositional analysis demonstrated significant differences between 1507xMON810xMIR162xNK603 maize and non-modified counterpart for 36 analytes (58%). In addition, 8 analytes exceeded the range of reference variation (equivalence outcome category II). The applicant discussed if alterations of each of those 8 analytes separately could have a negative impact of food and feed safety in term of nutrient value. Alteration of the other 28 analytes was ignored, as well as any interconnection between altered analytes. However, all analytes interact in terms of metabolite succession and biological function. The observed differences in 1507xMON810xMIR162xNK603 maize composition did not occur randomly but indicate a systemic upregulation of stress response pathways. The interconnections between altered analytes are discussed in the following. For simplification in the following the outcome type is written in brackets, a significant increase is marked with '+' and a significant decrease in marked with '-' (e.g. phenylalanine was identified as outcome type four and was significantly increased compared to non-modified counterpart, hence it is marked "phenylalanine (4+)"). Metabolites, which were not measured within composition analysis are marked as (nm)</p> <p>Shikimate- and phenylpropanoid pathway:</p>	<p>As described in section 3.4.2.6, the GMO Panel assessed all the significant differences between maize 1507 x MON810 x MIR162 x NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the four-event stack maize and the non-GM comparator and falling under category III/IV were identified.</p>

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		<p>Phenylalanine (4+) and tyrosine (2+) are synthesized via phrephenate metabolism within the shikimate pathway. As concentrations of both analytes were increased in 1507xMON810xMIR162xNK603 maize an upregulation of phrephenate metabolism should be considered. Phenylalanine (4+) and tyrosine (2+) are precursors for the phenylpropanoid pathway. The latter includes p-coumaric acid (2+) and ferulic acid (2+) (Dixon &amp; Paiva, 1995), as well as lignin (nm). Lignin was not measured but corresponds to ADF (2+) and NDF (2+). Another product of the phenylpropanoid pathway is salicylic acid (nm), which plays a major role during the plant response to abiotic stress, as well as in plant growth and development (Gunes et al., 2007). In particular growth stimulating effects of salicylic acid have been reported (Gunes et al., 2007). Analysis of agronomic traits of 1507xMON810xMIR162xNK603 maize demonstrated significant increase in plant height (4+) and ear height (2+) (CI: PHI-2012-023), supporting the assumption of increased salicylic acid. In conclusion there are good indications for upregulation of phenylpropanoid-pathway in 1507xMON810xMIR162xNK603 maize. Many products of the phenylpropanoid pathway are toxic or endocrine disruptive, hence harmful for human and animal health. The indicated upregulation of the phenylpropanoid pathway in 1507xMON810xMIR162xNK603 maize could lead to increased concentrations of harmful phenylpropanoids. Further analysis of such compounds should be considered to assess the safety for food and feed.</p> <p>Tocopherol-pathway:  Phenylalanine (4+) and tyrosine (2+) are precursors for the γ-tocopherol (4+) synthesis (Collakova and DellaPenna, 2003). Tocopherol is a strong antioxidant and is crucial for scavenging reactive oxygen species released during oxidative stress (Asensi-Fabado and Munne-Bosch, 2010). Y-tocopherol is more protecting than α-tocopherol. A shift in tocopherol composition towards γ-tocopherol occurs under oxidative stress</p>	
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		<p>(Kanwischer et al. 2005) and was also observed in 1507xMON810xMIR162xNK603 maize. Tocopherol belongs to antioxidant vitamins, which include also carotinoids (1), ascorbate (nm), thiamine (4+) and vitamin B6 (2+) (Asensi-Fabado and Munee-Bosch, 2010). Tocopherol interacts with the ascorbate-glutathione cycle, as the oxidized form of tocopherol can be regenerated by interaction with ascorbate and glutathione (Kanwischer et al. 2005). One intermediate of the ascorbate synthesis pathway is phytic acid (2+) (Lorence et al. 2004). The ascorbate-glutathione cycle is of major importance in plant defense to oxidative stress, hence this key metabolism should be analyzed in 1507xMON810xMIR162xNK603 maize.</p> <p>Cyanogenic glycosides pathway: Phenylalanine (4+) and tyrosine (2+), as well as valine (2+), leucine (4+) and isoleucine (4+) are precursors for the cyanogenic glycoside pathway (Poulton, 1990). Maize is a cyanogenic plant (Jones, 1998). Cyanogenic glycosides (nm) themselves are not toxic to mammals. However, they can generate hydrocyanic acid (HCN) when degraded by <math>\beta</math>-glycosidase, which is either endogenous to maize or produced by micro-organisms in the mammalian digestive tract (Jones, 1998). HCN is highly toxic. Alterations in cyanogenic glycosides of 1507xMON810xMIR162xNK603 maize need further investigation.</p> <p>Amino acids: Leucine (4+), isoleucine (4+) and valine (2+) belong to the group of branched-chain amino acids (BCAAs). They are coordinately regulated (Joshi et al. 2010). As a general response to abiotic stress plants accumulate free amino acids, especially BCAAs (Joshi et al. 2010). Upregulation of more than 70% of amino acids (including BCAAs) indicate increased stress in 1507xMON810xMIR162xNK603 maize. Manetti et al. (2006) identified significant changes in metabolites of primary nitrogen metabolism, in particular isoleucine, leucine and valine, in Bt maize grain expressing Cry1A gene. The link between the expression of Bt proteins</p>	
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		<p>and the upregulation of BCAAs should be further investigated in 1507xMON810xMIR162xNK603 maize.</p> <p>Vitamine B1 and oxylipin pathway:</p> <p>Vitamine B1 (4+) plays a fundamental role as an enzymatic cofactor in the above mentioned pathways and has been shown to have functions in response to abiotic and biotic stress in plants (Goyer 2010). The active coform of Vitamin B1, thiamine diphosphate (TDP), serves as a cofactor of acetolactate synthase (which catalyses the first step in the synthesis of BCAAs), transketolase (which is a key determinant in phenylpropanoid metabolism) and pyruvate dehydrogenase (which provides acetyl-coenzyme A and NADH for de novo fatty acid biosynthesis). Amongst others, fatty acids are involved in stress response in plants, hence their function correspond to the function of the above discussed metabolites. Plants respond to abiotic and biotic stress by remodeling membrane fluidity and by releasing <math>\alpha</math>-linolenic acid (2+) from membrane lipids (Upchurch 2008). Free linolenic acid is itself a stress signal and the precursor for oxylipin biosynthesis (Upchurch 2008, Blee 2002). Linolenic acid is metabolized via oxylipin pathway into jasmonic acid (nm), which plays a central role in plant development and plant defense. Within plant development jasmonic acid is – amongst many other functions - crucial for pollen development and increased pollen viability (McConn and Browse, 1996). Analysis of agronomic traits of 1507xMON810xMIR162xNK603 maize demonstrate increased pollen viability shape (2+) and increased pollen viability color (2+). This data support the thesis of upregulation of the oxylipin pathway in 1507xMON810xMIR162xNK603 maize. Within plant defense jasmonic acid in the oxylipin pathway is coordinately activated with the phenylpropanoid and the shikimate pathway in order to combat environmental stress (Alvarez et al. 2016). There are strong indications that all three pathways are upregulated in 1507xMON810xMIR162xNK603 maize,</p>	
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		<p>hence a systemic upregulation of stress response pathways needs to be considered.</p> <p>Impact of intended herbicide treatment</p> <p>Results of nutrient composition analysis indicate impact of intended herbicide application (i.e. mixture of glyphosate and glufosinate) on 1507xMON810xMIR162xNK603 plant metabolism.</p> <p>Alteration in nutrient composition increased at intended herbicide treatment (IHT) compared to conventional herbicide treatment (CHT) for all analytes corresponding with stress response pathways (i.e. all amino acids, ADF, NDF, pterulic acid, coumaric acid, linolenic acid, phytic acid) except vitamins. This is in line with literature reports where glyphosate was found to alter physiological processes in glyphosate resistant plants, including photosynthesis, mineral nutrition and oxidative events (reviewed by Gomes et al. 2014). In addition, glyphosate alters lignin and amino acid content (Zobiole et al. 2010a) as well as fatty acid composition of seeds (Zobiole et al. 2010b). As herbicide resistance is one of the desired traits of 1507xMON810xMIR162xNK603 maize the impact of herbicide treatment on the GMO deserves more attention. The impact of each herbicide should be analyzed separately and in combination and for different herbicide concentrations (including the highest concentration tolerated by 1507xMON810xMIR162xNK603 maize). Measurement of herbicide residues should be included to verify herbicide uptake, transport, accumulation and metabolism. Statistical analysis of results is required.</p> <p>Impact of genetic modification on plant metabolism:</p> <p>In summary nutrient composition analysis indicated systemic upregulation of stress-response pathways in 1507xMON810xMIR162xNK603 maize, which was most obvious at intended herbicide treatment. The reasons for this alteration need to be identified. In this respect the impact of genetic modification on plant metabolism should be elaborated. Most of the identified stress response pathways interact with the shikimate</p>	
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		<p>pathway, which includes the genetically modified cp4EPSPS. Upregulation of shikimate-pathway could be induced either due to the increased concentrations of total EPSPS (i.e. natural EPSPS plus transgenic cp4EPSPS) or due to increased enzymatic activity of cp4EPSPS compared to natural EPSPS. The shikimate-pathway corresponds with the phenylpropanoid-pathway. Literature reports demonstrate an alteration in phenylpropanoid-pathway in glyphosate-resistant soybean (Zonetti et al. 2011) and Bt cotton (Li et al. 2015), indicating that phenylpropanoid pathway is susceptible for alterations due to genetic engineering. Bt protein expression was found to correspond with upregulation of oxylipin and phenylpropanoid pathways both in Bt rice and Bt maize MON810, indicating a link between Bt expression and internally induced chemical defense systems (Liu et al. 2012, Feng et al. 2007). In particular, the interaction between Bt expression, jasmonic acid (as part of the oxylipin pathway) and direct defense proteins (synthesized via phenylpropanoid-pathway) was analyzed for MON810 (Feng et al. 2007). In addition alterations in amino acid composition have been demonstrated for Bt rice (Liu et al. 2012). The link between Bt-expression and stress response should be further investigated in 1507xMON810xMIR162xNK603 maize.</p> <p>Phosphomannose-isomerase (PMI) is an enzyme involved in the ascorbate synthesis (Lorence et al. 2004). Ascorbate is of major importance to combat oxidative stress in plants. Effects of PMI on ascorbate synthesis should be analysed.</p> <p>The PAT enzyme belongs to the functional group of acetyltransferases. PAT transfers an acetyl-group to the free NH<sub>2</sub> group of glufosinate, causing the inactivation of the herbicide. Donor of the acetyl-group is Acetyl-CoA, which is the most important cofactor for many other enzymes in plant metabolism. Competition between PAT and other acetyltransferases for Acetyl-CoA is likely to occur. The impact of additional demand</p>	
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		<p>of Acetyl-CoA for pant metabolism needs further investigation in 1507xMON810xMIR162xNK603 maize.</p> <p>Conclusions:</p> <p>In conclusion there are good indications that plant defence pathways were upregulated in 1507xMON810xMIR162xNK603 maize, indicating that the genetic modifications stress the plant under normal conditions. This explanation is backed by literature results demonstrating increased oxidative stress in glyphosate-resistant soy (Ayyadurai &amp; Deonikar 2015, Arruda et al 2013, Barbosa et al. 2012) and increased stress response in Bt rice (Liu et al. 2012) or Bt maize (Feng et al. 2007). We recommend to investigate alterations in stress response in 1507xMON810xMIR162xNK603 more carefully, therefore analyzing key metabolites and enzymes of the above identified pathways. In addition metabolome, transcriptome and proteome analysis are recommended to allow for a broader and more comprehensive identification of altered pathways. The impact of additional stressors, such as intended herbicide treatment and environmental conditions, on stress response in 1507xMON810xMIR162xNK603 should be investigated in detail. Field studies should be therefore supplemented with glasshouse studies, which allow for more controlled investigations of certain stressors. Unintended effects due to the interaction of transgenic proteins/enzymes with metabolic pathways need to be analysed. Only after comprehensive analysis of metabolic alterations in 1507xMON810xMIR162xNK603 maize a risk assessment can be conducted.</p> <p>Alvarez, A. et al. (2016). <i>Frontiers in Plant Science</i>, 22, 328. doi: 10.3389/fpls.2016.00328</p> <p>Arruda, S.C.C. et al. (2013). <i>Journal of Proteomics</i>, 93, 107-116.</p> <p>Asensi-Fabado, M. and Munne-Bosch, S. (2010). <i>Trends in Plant Science</i>, 15, 582-592.</p> <p>Ayyadurai, V.A.S. and Deonikar, P. (2015). <i>Agricultural</i></p>	
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		<p>Sciences, 6, 630-662.</p> <p>Barbosa, H.S. et al. (2012). Analytical and Bioanalytical Chemistry, 402, 299-314.</p> <p>Bleé, E. (2002). Trends in Plant Science, 7, 315-321.</p> <p>Collakova, E. and DellaPenna, D. (2003). Plant Physiology, 133, 930-940.</p> <p>Dixon, R.A. and Paiva, N.L. (1995). The Plant Cell, 7, 1085-1097.</p> <p>Feng, Y-j., Wang, J-w., Luo, S-m. (2007). Agricultural Sciences in China, 6, 1456-1462.</p> <p>Gomes, M.P. et al. (2014). Journal of Experimental Botany, 65, 4691-4703.</p> <p>Goyer, A. (2010). Thiamine in plants: Phytochemistry, 71, 1615-1624.</p> <p>Gunes A, et al. (2007). Journal of Plant Physiology 164, 728–736.</p> <p>Jones, D.A. (1998). Phytochemistry, 47, 155-162.</p> <p>Joshi, V., Joung, J-G., Fei, Z. (2010). Amino Acids, 39, 933-947.</p> <p>Kanwischer, M. et al. (2005). Plant Physiology, 137, 713-723.</p> <p>Li, X., Ding, C., Wang, X., Liu, B. (2015). Scientific Reports, 5: 8739, Doi: 10.1038/srep08739</p> <p>Liu, Z. et al. (2012). International Journal of Biological Sciences, 8, 953-963.</p> <p>Lorence, A. et al. (2004). Plant Physiology, 134, 1200-1205.</p> <p>Manetti, C. et al. (2006). Journal of Experimental Botany, 57, 2613-2625.</p> <p>McConn, M., Browse, J. (1996). The Plant Cell, 8, 403-416.</p> <p>Poulton, J.E. (1990). Plant Physiology, 94, 401-405.</p> <p>Upchurch, R.G. (2008). Biotechnology Letters, 30, 967-977.</p> <p>Zobiolo LHS, et al. (2010a). Acta Physiologiae Plantarum 32, 831–837.</p> <p>Zobiolo LHS, et al. (2010b). Journal of Agricultural and Food Chemistry 58, 4517–4522.</p> <p>Zonetti, P. et al. (2011). Acta Scientiarum. Agronomy, 33, 291-295.</p>	
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Germany	BfN	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>Results of the agronomic and phenotypic characteristics support the assumption of altered physiological processes in 1507xMON810xMIR162xNK603 maize. In particular observed alterations in plant height and pollen variability correspond to alterations in nutrient analysis (see above A.3.3).</p>	<p>Two independent sets of field trials were received: North America (2012) and North America (2015); both sets were considered, although for different purposes. Compositional data were provided only for the field trials in 2012. Agronomic-phenotypic data were measured in both sets, but they were assessed based only on data from the field trials in 2015, as those in 2012 did not include the measurement of yield components (yield and kernel weight) and therefore were considered incomplete. Suitability of materials and representativeness of the receiving environments were assessed for both years. Early stand count and yield for the four-event stack maize (treated) showed significant differences with respect to the non-GM comparator and fell into equivalence category III. Whether the differences can lead to an environmental adverse effect is considered in Section 3.4.4. of the scientific opinion.</p>
Germany	BfN	II.1.4 Toxicology	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The toxicological assessment by the applicant is mainly based on the expression of the new proteins, but not on potential unintended effects deriving from genetic transformation. Potential alterations of metabolic pathways were not considered although results of nutrient analysis indicate upregulation of stress defense pathways in 1507xMON810xMIR162xNK603 maize (see A.3.3). Such alterations could result in accumulation of secondary plant products, which are either toxic or endocrine disruptive (see A.3.3). Therefore, the BfN would welcome long-term chronic studies including reproduction and offspring health. Since it has been shown that the most severe changes in plant analytes appear under herbicide treatment (see A.3.3) the feeding studies include feed derived from 1507xMON810xMIR162xNK603 maize treated and non-treated with intended herbicides. The broiler feeding study provided by the applicant did not include toxicological parameters and hence is not suitable for toxicological assessment.</p>	<p>The GMO Panel takes note of the comment. Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of maize 1507 x MON810 x MIR162 x NK603 have been identified. Therefore, additional animal studies on food/feed derived from the four-stack are not necessary.</p>

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Germany	BfN	II.4 Post-market monitoring on the genetically modified food or feed	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The data provided to show the human and animal safety of 1507xMON810xMIR162xNK603 maize on the basis of its substantial equivalence to conventional maize (except for the introduced trait) are not sufficient. Therefore, a post-market monitoring for food and feed is required.</p> <p>The applicant is further requested to explain how the PMM of 1507xMON810xMIR162xNK603 maize in mixed GMO commodities imported, processed or used for food/feed is realised. This is requested because the monitoring of a GMO must be carried out on a case-by-case basis (Directive 2001/18/EC) with regard to species characteristics, modified traits, the intended use and the degree of exposition. Specific GM product quantities should be provided to estimate the degree of exposition. In case of mixed commodities, according to the precautionary principle, each imported and processed commodity must be assumed to contain any in EU approved GM maize and consequently all parameters identified for the different GM maize products should then be monitored.</p>	As reflected in section 3.6.1. of the EFSA scientific opinion, the GMO Panel concluded that the four-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the non-GM reference varieties tested. Therefore, post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary.
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Germany	BfN	II.5.3.4 Interactions of the GM plant with non-target organisms (NTOs)	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>Exposure analysis  The scope of the application includes processing and the use for food and feed purposes. The main exposure route therefore will result from waste produced during processing and the use of the GMO as food and feed. Although the applicant briefly addressed the issue the exposure analysis in the dossier is incomplete and not backed by any measurements of the Bt proteins in manure or waste products.</p> <p>For Bt proteins an exposure route via manure from cattle fed with Bt maize has been demonstrated (Gruber et al. 2011; Gürtler et al. 2010). Paul et al. (2010) observed that 44% of the immunoreactive Cry1Ab from MON810 present in feed was transferred to the feces (Paul et al. 2010) while 34% of the Cry1Ab protein levels in feed could be detected in liquid manure (Gruber et al. 2011). As Gruber et al. (2011) demonstrated Cry1Ab is relatively stable in liquid manure (decrease of 49% in 24 weeks). The bioactivity of Cry proteins in wastewater or manure is unknown as no bioassays have been carried out so far. Based on the above finding it is likely that all three Bt proteins present in the GMO (Cry1Ab, Cry1F, Vip3A) will contribute to an environmental exposure for which effects cannot be assessed as both quantitative data on the exposure and data on the hazard for soil and water organisms are lacking.</p> <p>Effects on non-target organisms  Based on the exposure analysis the applicant should provide data on the ecotoxicity of 1507xMON810xMIR162xNK603 maize to assess possible effects on non-target organisms and subsequent effects on biogeochemical processes. Little information on combinatorial effects between the different Bt proteins (or Bt proteins and other components such as HR) exist. As the outcome cannot</p>	<p>Considering the scope of application EFSA-GMO-NL-2015-127, which excludes cultivation, the environmental risk assessment (ERA) of maize 1507 x MON810 x MIR162 x NK603 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize 1507 x MON810 x MIR162 x NK603 grains during transportation and/or processing (EFSA GMO Panel, 2010).</p> <p>Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled GM grains is limited and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions with non-target organisms are not considered a relevant issue by the GMO Panel. that may occur between the <i>Bt</i> proteins expressed in this GM maize will not alter this conclusion.</p>
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		<p>be predicted a priori (Hilbeck &amp; Otto 2015) tests are necessary to address this issue.</p> <p>Although the scope of the current application does not include cultivation, import and processing of the GMO may lead to environmental exposure via waste or feces resulting from the use of the GMO as food or feed. Consequently soil and water organisms are the most likely groups which will be exposed to the novel proteins. Exposure routes, functional groups and test species should be selected according to an ecological test strategy (Hilbeck et al. 2008, 2014). Having collected data on the ecotoxicity the risk assessment should be updated including possible effects on soil and water organisms. A representative set of organisms with a high probability of exposure should be tested as Bt toxins are less specific than previously assumed and their sensitivity is difficult to predict (van Frankenhuyzen 2009, 2013; Hilbeck and Otto 2015). We recommend including water organisms in ecotoxicity testing. Several recent publications point at the presence of Cry Proteins and/or genes in aquatic systems and raise concerns about the safety of plant expressed Cry-Proteins to aquatic organisms (Bøhn et al. 2008, 2010, 2016; Douville et al. 2005, 2008; Prihoda &amp; Coats, 2008; Rosi-Marshall et al. 2007). Bøhn,T., Primicerio,R., Hessen,D.O. &amp; Traavik,T. (2008). Arch Environ Contam Toxicol, 55 (4), 584-592. Bøhn,T., Traavik,T., Primicerio,R. (2010). Ecotoxicology, 19, 419-430. Bøhn,T., Macagnan Rover,C., Semenchuk,P.R. (2016). Food and Chemical Toxicology, 91, 130-140. Douville,M., Gagné,F., Masson,L., McKay,J. &amp; Blaise,C. (2005). Biochemical Systematics and Ecology, 33, 219-232. Douville,M., Gagné,F. &amp; Blaise,C. (2008). Ecotoxicology and Environmental Safety, 72 (1), 17-25. Gruber,H., Paul,V., Guertler,P., Spiekers, H., Tichopad, A., Meyer, H. H. D. &amp; Müller, M. (2011). Journal of Agricultural &amp; Food Chemistry 59, 7135–7144. Gürtler, S.P., Paul, V., Steinke, K., Wiedemann, S.,</p>	
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		<p>Preißinger, W., Albrecht, C., Spiekers, H., Schwarz, F. J. &amp; Meyer, H. H. D. (2010). <i>Livestock Science</i>, 131, 250-259.</p> <p>Hilbeck, A. &amp; Otto, M. (2015). <i>Frontiers in Environmental Science</i>, 3, 71.</p> <p>Hilbeck, A., Weiss, G., Oehen, B., Römbke, J., Jänsch, S., Teichmann, H., Lang, A., Otto, M., Tappeser, B. (2014) <i>Ecological Indicators</i>, 36, 367-381.</p> <p>Hilbeck A., Jänsch, S., Meier M., Römbke J. (2008). Federal Agency for Nature Conservation, Bonn - Bad Godesberg: 287 pp. (BfNSkript 236)  <a href="http://www.bfn.de/fileadmin/MDB/documents/service/skript236.pdf">http://www.bfn.de/fileadmin/MDB/documents/service/skript236.pdf</a></p> <p>Paul, V., Guertler, P., Wiedemann, S., and Meyer, H. H. (2010). <i>Transgenic Research</i>, 19 (4), 683-689.</p> <p>Prihoda, K. R. &amp; Coats, J. R. (2008) <i>Environmental Toxicology and Chemistry</i>, 27, 793-798.</p> <p>Rosi-Marshall, E. J., Tank, L. J., Royer, T. V., Whiles, M. R., Evans-White, M., Chambers, C., Griffiths, N. A., Pokelsek, J., Stephen, M. L. (2007). <i>Proceedings of the National Academy of Science USA</i>, 104, 16204-16208.</p> <p>van Frankenhuyzen, K. (2009). <i>Journal of Invertebrate Pathology</i>, 101, 1-16.</p> <p>van Frankenhuyzen, K. (2013). <i>Journal of Invertebrate Pathology</i>, 114, 76-85.</p>	
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Germany	BfN	II.6 Post-Market Environmental Monitoring Plan (PMEM)	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The scope of this application is for import, processing, and all uses for food and feed. The applicant provides an environmental monitoring plan, which remains very general. The structure of the monitoring plan has to be provided in accordance with EFSA Journal (2011). The monitoring plan has to be elaborated in more detail in order to meet the following requirements:</p> <ul style="list-style-type: none"> <li>- Provision of a fully specified list of monitoring parameters.</li> <li>- Application of standardised sampling methodologies: A basic prerequisite for comparing GMO monitoring data is the use of appropriate standard detection or analytical methods. Several standards specific for GMO monitoring are provided by the Association of German Engineers (VDI). They are available under <a href="http://www.vdi.eu/engineering/vdi-standards/">http://www.vdi.eu/engineering/vdi-standards/</a>.</li> <li>- Elaboration of a sampling concept.</li> <li>- In case of monitoring data being collected by external persons or institutions other than the applicant, binding agreements/contracts with third parties are requested which clearly determine what data are provided and how these data are made available.</li> <li>- Elaboration of the methods of data analysis including the statistical methods.</li> <li>- Application of the concept of adverse effects and environmental damages: Adverse environmental effects can only be determined if they are related to certain relevant subjects of protection (Bartz et al. 2009). The subject of protection is damaged if it is significantly adversely affected. The identification of a significant adverse effect should consider both its intensity (e.g. extent of loss) and the value of the impaired subject of protection (e.g. high value of protected species).</li> </ul> <p>The monitoring should be run in regions, where 1507xMON810xMIR162xNK603 maize will be</p>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.</p>
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		<p>transported, stored, packaged, processed or used. In case of substantial losses and spread of 1507xMON810xMIR162xNK603 maize, all receiving environments need to be monitored.</p> <p>The time period of monitoring needs to be sufficient to detect delayed or long-term adverse effects. Therefore it may be necessary to extend the monitoring regarding certain parameters beyond the period of consent.</p> <p>Since traders may commingle 1507xMON810xMIR162xNK603 maize with other commercial GM maize imported, processed or used for food/feed, the applicant is requested to explain how the monitoring will be designed to distinguish between potential adverse effects caused by 1507xMON810xMIR162xNK603 maize and those caused by other GM maize.</p> <p>The Federal Agency for Nature Conservation is of the opinion that a detailed monitoring plan has to be provided before consent may be given.</p> <p>There are gradual differences in the predictability among effects and therefore gradual transitions between case-specific monitoring and general surveillance. It is therefore necessary to include the option of investigating similar parameters in case-specific monitoring, in general surveillance, or in both simultaneously. Consequently, monitoring requirements are listed under both categories.</p> <p>Bartz, R., Heink, U. &amp; Kowarik, I. (2009): Conservation Biology 24 (3), 675–681. DOI: 10.1111/j.1523-1739.2009.01385.x</p> <p>EFSA (2011). Scientific opinion. EFSA Journal, 9(8), 2316, 40 pp</p>	
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Germany	BfN	II.6.1 Interplay between Environmental Risk Assessment, Risk Management and PMEM	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The information necessary to conclude on the ERA is partly missing. Thus, the safety of 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize cannot be fully assessed. Depending on those results the conclusions concerning case-specific monitoring may need to be revised.</p>	<p>The GMO Panel considered that the information submitted by the applicant on application EFSA-GMO-NL-2015-127 was sufficient to conclude on the environmental risk assessment (ERA) of maize 1507 x MON 810 x MIR162 x NK603.</p> <p>As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.</p>
Germany	BfN	II.6.2 Case Specific Monitoring (strategy, method and analysis)	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>We do not share the opinion of the applicant that a case-specific monitoring is not necessary. Case-specific monitoring has to focus on pathways, where 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize or material containing 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize enter the environment including sewage water, waste material or by-products which occur during processing or use of 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize as food and feed. The applicant is requested to provide an appropriate case-specific monitoring plan comprising at least the following elements:</p> <p>i.) spillage or loss of 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize during transport, storage, packaging, processing and use,</p> <p>ii.) potential spread and persistence of 1507xMON810xMIR162xNK603/1507xMON810xMIR162xNK603 maize, if spillage or loss of viable 1507xMON810xMIR162xNK603 maize occurs,</p> <p>For parameters in i.) – ii.), the use of the following methods is recommended (www.vdi.de):</p> <ul style="list-style-type: none"> <li>o VDI-Guideline 4330 Part 10 "Floristic mapping of genetically modified plants their crossing partners and their hybrid offspring"</li> <li>o VDI-Guideline 4330 Part 5 "Guideline for the</li> </ul>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.</p> <p>As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.</p>



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		<p>collection and preparation of plant samples for molecular biological analysis”</p> <p>iii.) exposure of the different Bt proteins to the environment e.g. via sewage water, waste material, manure or by-products which occurs during processing or use of non-viable material of the GMO as food/feed</p> <p>iv.) environmental effects such as spread, persistence and accumulation of the different Cry proteins in other organisms and environmental media</p> <p>If spread, persistence or accumulation of 1507xMON810xMIR162xNK603 maize or 1507xMON810xMIR162xNK603 maize products in the receiving environment occur, further observations of possible impacts on organisms, food chains and habitats in the specific environment are required. 1507xMON810xMIR162xNK603 maize expresses three different Bt toxins. Furthermore 1507xMON810xMIR162xNK603 maize may enter the environment together with other approved GM maize lines containing different Bt proteins. Therefore, a special focus should be on potential effects on the environment based on the combination of several Bt-toxins.</p> <p>The control of adventitious maize plants and clean up measures are proposed to control spillage of viable plant material during transport, storage, packaging or processing. The Federal Agency for Nature Conservation is of the opinion, that these risk management measures should be confirmed as mandatory. Furthermore, the efficacy of the implemented risk management measures should be monitored during case specific monitoring (EFSA 2011).</p> <p>VDI (2011). VDI Guidelines: monitoring the ecological effects of genetically modified organisms. Genetically modified plants. <a href="http://www.vdi.de/42479.0.html">http://www.vdi.de/42479.0.html</a></p> <p>EFSA (2011). Scientific opinion. EFSA Journal, 9(8), 2316, 40 pp.</p>	
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Germany	BfN	II.6.3 General Surveillance (strategy, method)	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The applicant states that the general surveillance will be based on information gathered from the existing networks of COCERAL, UNISTOCK and FEDIOL. Data shall be collected by local factory operators. Communication with local operators will be delegated to EuropaBio. An important tool for information exchange will be the website hosted by EuropaBio. It is not clear, how EuropaBio will inform local operators about their surveillance function and how it will be assured that local operators in duty for general surveillance show the necessary skills to detect environmental impacts of 1507xMON810xMIR162xNK603 maize. Therefore, the applicant is requested</p> <ul style="list-style-type: none"> <li>i.) to name the national and local organisations and factories involved in the monitoring,</li> <li>ii.) to prove that a sufficient number of local operators agree to contribute to the general surveillance, to provide a schedule with all relevant observation objects to be monitored,</li> <li>iii.) to explain how local operators will be instructed and trained for conducting the general surveillance, to verify the necessary skills and expertise of local operators to detect adverse environmental impacts.</li> </ul> <p>In case the suggested operators are not capable to cover all relevant observation objects, further monitoring systems have to be established. The applicant does not suggest operators further down the food chain to be involved in the process of monitoring. We do not approve this, because processed material may also be a cause of adverse effects. Therefore, the applicant is requested to involve also operators further down the food chain in the process of monitoring.</p> <p>The general surveillance plan has to focus on possible pathways how 1507xMON810xMIR162xNK603 maize can get into the broader environment and how</p>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.</p>
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			<p>unforeseen adverse effects on human health and the environment can be linked to the dispersal and use of 1507xMON810xMIR162xNK603 maize. Beside the implementation of management and safety standards, the applicant is requested to provide an appropriate general surveillance plan comprising the monitoring of spillage or losses of 1507xMON810xMIR162xNK603 maize during transport, storage, packaging, processing and use, potential spread and persistence of 1507xMON810xMIR162xNK603 maize.</p> <p>1507xMON810xMIR162xNK603 maize may enter the environment together with other approved GM maize lines. Therefore, a special focus should be on possible combined effects.</p> <p>Annex 1 of the monitoring plan is missing.</p>	
Germany	BfN	II.6.4 Reporting the results of PMEM	<p>Additional comments by the Federal Agency for Nature Conservation:</p> <p>The applicant is required to report on the results of the monitoring including all issues of case-specific monitoring and general surveillance on an annual basis. Raw data have to be made available.</p> <p>The monitoring report should also deliver detailed information on</p> <ul style="list-style-type: none"> <li>i) actual volumes of 1507xMON810xMIR162xNK603 maize imported into the EU,</li> <li>ii) the ports and silos where shipments of 1507xMON810xMIR162xNK603 maize were unloaded,</li> <li>iii) the processing plants where 1507xMON810xMIR162xNK603 maize was transferred to,</li> <li>iv) the amount of 1507xMON810xMIR162xNK603 maize used on farms for feed, and</li> <li>v) transport routes of 1507xMON810xMIR162xNK603 maize.</li> </ul>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 × MON 810 × MIR162 × NK603.</p>

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Germany	BVL (CA Germany)	II.1 Hazard identification and characterisation	<p>The scope of application EFSA-GMO-NL-2015-127 covers import and processing of maize 1507xMON810xMIR162xNK603 including all feed and food products containing, consisting of, or produced from the genetically modified maize 1507xMON810xMIR162xNK603. Cultivation is not covered by this application.</p> <p>The application also seeks authorisation for the placing on the market of the following sub-combinations of events, independently of their origin, for the commercial uses detailed above:  MON810xMIR162xNK603, 1507xMIR162xNK603, 1507xMON810xNK603, 1507xMON810xMIR162, 1507xMON810, 1507xMIR162, 1507xNK603, MON810xMIR162, NK603xMON810, and MIR162xNK603. All four single events and two double stacks (1507xNK603, NK603xMON810) have already been risk assessed and are authorised for import and use in feed and food in the EU. Cultivation is not covered by this application.</p> <p>Taken as a whole, the Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion, that the entirety of available data supports the conclusion that maize 1507xMON810xMIR162xNK603 is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended use. The same applies for all possible sub-combinations. Nevertheless, completion on some points of the dossier is recommended (see specific comments). In addition, the provided monitoring plan is incomplete at this stage and needs further elaboration for implementation.</p>	The EFSA GMO Panel thanks Germany for the comment.
Germany	BVL (CA Germany)	II.1.2.2 Information relating to the genetically modified plant	<p>Information on the expression of the insert(s):  No data for protein expression in sub-combinations with exception of NK603xMON810 and 1507xNK603 (link to other applications) were submitted. The applicant should adequately substantiate why there is no need for experimental data to be obtained for other sub-combinations.</p>	The GMO Panel has assessed all single events and the higher stack. The information contained in the application was considered sufficient to conclude on the safety of the subcombinations of the events found in the four-event maize stack, in line with the IR 503/2013 and the EFSA GMO Panel subcombinations strategy document (Minutes of the GMO Panel 115 <sup>th</sup> Plenary meeting, 17-18 May 2017, Annex 1, available at

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				<a href="https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf">https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf</a> ).
Germany	BVL (CA Germany)	II.1.4.1 Testing of newly expressed proteins	With intent to demonstrate the unlikelihood of the interaction between Cry1F and Cry1Ab the applicant refers to the application EFSA-GMO-DE-2010-86. However the study on the interaction of the Cry1F, Cry1Ab and VipAa20 proteins on target pests could not be identified in the mentioned application. We propose that appropriate data and information should be clearly represented within the application documents of dossier 1507xMON810xMIR162xNK603.	The GMO Panel takes note of the comment. On the basis of the known biological function of the individual newly expressed proteins, there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize 1507 x MON810 x MIR162 x NK603. Interactions of the GM plant with the target and non-target organisms are addressed in sections 3.4.4.3 and 3.4.4.4 of the scientific opinion.
Germany	BVL (CA Germany)	II.1.6.2 Nutritional assessment of the genetically modified feed	Although feed intake data were collected, they were not identified within the study PHI-2012-012 (Annex 24) and should be provided for the sake of completeness.	The GMO Panel takes note of the comment.
Germany	BVL (CA Germany)	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	The import documents should indicate that maize 1507xMON810xMIR162xNK603 has not been approved for cultivation by the EC. In addition to the intended GM labelling, a clear labelling of maize 1507xMON810xMIR162xNK603 indicating the tolerance to glyphosate and glufosinate-ammonium herbicides is recommended. Furthermore, appropriate measures have to be taken during transport, storage, and processing to avoid unintended release of germinable maize kernels into the environment. In this context, the applicant should inform all parties involved in the handling and processing of maize 1507xMON810xMIR162xNK603 about avoidance and control of spillage.	Labeling is not in the remit of the GMO Panel. Similarly, monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603. As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.

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Germany	BVL (CA Germany)	II.6 Post-Market Environmental Monitoring Plan (PMEM)	The monitoring plan is acceptable, but needs further elaboration for implementation. Therefore, the applicant is recommended to revise the monitoring plan during the initial implementation phase (after consent is given) and present this revised monitoring plan together with a first report one year after consent is given to be reassessed.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA.
Germany	BVL (CA Germany)	II.6.2 Case Specific Monitoring (strategy, method and analysis)	According to the risk assessment, no adverse effects on the environment or human health were identified or were expected. Therefore, there is no necessity for a case-specific monitoring.	As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.
Germany	BVL (CA Germany)	II.6.4 Reporting the results of PMEM	A report on GS activities on an annual basis is sufficient. Reporting should refer to the format introduced by the Commission Decision 2009/770/EC. The applicant is requested to state how the monitoring results will be published.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA.
Germany	BVL (CA Germany)	II.6.3 General Surveillance (strategy, method)	<p>Approach</p> <p>The monitoring plan does not relate the monitoring activities to relevant protection goals. Even more it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter, because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate food and feed surveillance in coordination with the competent authorities. Information about the use of the product in food and feed could deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Therefore, the applicant should specify monitoring activities in the field of human and animal health. He should describe in detail how animal and human health surveillance is integrated in the monitoring plan.</p> <p>The strategy of General Surveillance is mainly based</p>	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA.

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		<p>on the involvement of importers, traders, silo operators and processors coordinated by EuropaBio. The applicant will inform the selected networks of operators about market release of GM plant products and will remind them to report on 'any unanticipated adverse effect'. He stated that these third parties have to follow legal obligations of food and feed hygiene (HACCP). Nevertheless, the role and interplay of all actors on behalf of recording, analysis and evaluation of monitoring data needs more transparency.</p> <p>Identification of existing networks  The applicant should consider whether other existing monitoring networks might be used in particular in the field of human and animal health. In such a case, the selection and evaluation process should be described in detail.</p> <p>In general, other sources of information e.g. peer-reviewed publications or on going research should be taken into account. However, the applicant should describe in detail how he would consider this information within General Surveillance.</p>	
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Hungary	Ministry of Agriculture	II.1.2.1 Information relating to the genetic modification	<p>1.2.1.2 The 1507xMON810xMIR162xNK603 maize contains the Cry1F (a synthetic version of truncated cry1F gene of <i>B. thuringiensis</i> subsp. <i>aizawai</i>) gene, the PAT (the plant-optimised glufosinate-ammonium tolerance gene) gene, the Cry1Ab (a variant of Cry1Ab1 protein from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>) gene, the Vip3Aa20 (a modified version of the native vip3Aa1 gene from <i>Bacillus thuringiensis</i> strain AB88 and differs from the vip3Aa19 gene by two nucleotides) gene, the PMI and CP4 EPSPS (plant optimized) genes.</p> <p>It is stated, that „the transgenic proteins expressed in 1507xMON810xMIR162xNK603 maize have a history of safe consumption“. Hungarian experts do not agree with this statement. Those gene and proteins have been modified and/or optimised to be expressed in plants and are different from the native proteins. They also have different regulatory elements attached to them. Neither the native nor the transgenic proteins were used as food or feed before. Although the Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been a part of the food/feed supply for about 10 years, it cannot be considered as “history”. In addition, there is no way to know if they have/had any harmful effect(s), since no one knows who has consumed what transgenic proteins, when and in what amounts. Therefore, it cannot be stated that those proteins were consumed in the EU for years without incidents. It is somewhat surprising that the bioinformatic evaluation of neither the Cry1F, nor the Cry1Ab, or the Vip3Aa20 protein sequence in 1507xMON810xMIR162xNK603 maize indicated any biologically relevant sequence similarity to “allergens, toxins, or other biologically active proteins” or “to any known or putative toxins”, since they should show similarities at least to the native cry toxins.</p>	<p>The GMO Panel takes note of the comment. (Please, refer to section 3.4.3.3 of the scientific opinion).</p> <p>The applicants use toxin databases which are internally developed. The databases are usually filtered to remove Cry proteins to avoid self-identification of Cry protein encoded in the events. The databases are validated using a self-identification step by random selection of a sequence from the database and by verifying that the search can indeed identify the randomly selected protein.</p>
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Hungary	Ministry of Agriculture	II.1.2.2 Information relating to the genetically modified plant	<p>1.2.2.1 It is stated that the presence of the promoters CaMV 35S in 1507xMON810xMIR162xNK603 maize is not a problem, since "CaMV is naturally present on many vegetables and it is likely that humans have had long exposure to the virus". This statement is misleading. The native CaM virus is covered by a protein coat, to which humans have no intestinal receptors for. However, the naked 35S promoter DNA sequence from CaM virus is operational in several species, including human cells. No one can tell if the ingestion of the promoter has any effect on humans or animals, since no such experiment has been performed.</p> <p>The CP4 EPSPS protein in 1507xMON810xMIR162xNK603 confers tolerance to the application of glyphosate herbicide. The question arises if the level of glyphosate (and of glufosinate) and its/(their) metabolites in the maize kernels and other parts of the plant (such as silage) are in the range permitted or are higher. Therefore, Hungarian experts respectfully suggest measuring the levels of these chemicals in every shipment of 1507xMON810xMIR162xNK603, and its relevant sub-stacks, when received by the EU.</p> <p>1.2.2.2 1507xMON810xMIR162xNK603 maize contains a copy of the intact gene from the expression cassette and a truncated cry1F fragment of 335 bp located at the 5' end, a DNA fragments, including incomplete sequences derived from the pat gene, the maize ubiquitin promoter and ORF25 terminator of Agrobacterium, found adjacent to the inserted main fragment; the cry1Ab gene derived from Bacillus thuringiensis subsp. kurstaki; a single copy of the vip3Aa20 and pmi genes, two copies of the maize polyubiquitin promoter (in addition to the endogenous polyubiquitin promoters) corresponding to the two copies of the promoter present in plasmid pNOV1300 used for transformation, one copy of the NOS terminator; and a copy of the a 5-enolpyruvylshikimate-3-phosphate synthase (epsps)</p>
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		<p>gene from <i>Agrobacterium</i> sp. strain.</p> <p>According to Hungarian experts to prove the stability of all those inserts, additional data are needed, such as the full sequences of the respective genomic regions. Those data should be submitted to establish the molecular identity of the inserts in 1507xMON810xMIR162xNK603 maize and the parental GM lines. The data submitted cannot identify minor alterations in the inserts, such as the single nucleotide polymorphisms (SNPs), which can be introduced during the breeding process and can affect the function of transgenic components.</p> <p>1.2.2.3 The Vip3Aa20 protein levels appear to be very different in IHT 1507xMON810xMIR162xNK603 maize (17 – 99) and in CHT 1507xMON810xMIR162xNK603 maize (14 – 280), as well as in CHT MIR162 maize (45 – 180). Based on these, how can one compare the stack with the appropriate event(s)?</p> <p>Although the inserts are present at different genetically loci, and the likelihood of molecular interactions between the different inserts is low, the interactions between the transgenic gene products, the individual proteins, cannot be excluded, but this interaction was not examined.</p> <p>1.2.2.4 The genetic stability of the actual 1507xMON810xMIR162xNK603 maize, at least for three generations, should be presented in the Dossier. Although the inserts are present at different genetically loci, and the likelihood of molecular interactions between the different inserts is low, there might be interactions between the transgenic gene products, the individual proteins. For example, it is feasible to conclude, that the expression of multiple cry proteins has an additive effect not only on plant protection but on target- and none target organisms, as it is described in the scientific literature. It is also likely that those proteins have multiple receptors and different effects/modes of actions in vivo on humans and animals consuming those cry proteins simultaneously, as it has been demonstrated by Bohn</p>	<p>In addition to the Southern analyses presented, the integrity of the inserts has been analysed by sequencing data provided upon EFSA's request (additional information clock2, 03/05/2016), and according to the Regulation (EU) No 503/2013 and the EFSA guidance for the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011), for all the individual events in the stack.</p> <p>The GMO Panel considers that the quality and results of the whole set of Southern analyses and sequencing data presented are considered sufficient to conclude on the maintenance of the structure of the inserts of the single events in the stack.</p> <p>The GMO Panel concludes that there is no indication of an interaction that may affect the integrity of the events or the levels of the newly expressed proteins in this stack.</p> <p>Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize 1507xMON 810xMIR162xNK603 grains is limited, and</p>
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			<p>et al (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) <i>Daphnia magna</i> negatively affected by chronic exposure to purified Cry-toxins. <i>Food and Chemical Toxicology</i> 91: 130-140). It was concluded that Cry-toxins in combination indicate alternative modes-of-action. The authors suggested that 'stacked events' may have stronger effects on non-target organisms, and that further studies are need to be done on the combinatorial effects of multiple Cry-toxins and herbicides that co-occur in the environment.</p> <p>1.2.2.5 A study conducted on human subjects fed on genetically modified soybean has shown that a proportion of the full length of the plant transgene does survive passage through the human gastrointestinal tracts, and evidence suggests that gene transfer actually occurred between GM soybean and intestinal micro-flora during the experiments (Netherwood et al., 2004). Indeed, the study has shown that the full length of the transgene, although in small quantities, survived digestion and could be detected from samples of microbes taken from the ileostomy bag (from microbes resident in the gut). Therefore, the possibility of horizontal gene transfer from the GM plants to gut microbes is quite likely in human or in animals. Mammals have been shown to take up dietary DNA from the gastrointestinal tract (Rizzi et al., 2012).</p>	<p>ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry and Vip proteins will not alter this conclusion. Genomic DNA can be a component of food/feed products derived from maize. 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 domesticated 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, chromosomally-located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).</p>
Hungary	Ministry of Agriculture	II.1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects	<p>1.2.3 The expression of multiple cry proteins has an additive effect not only on plant protection but on target- and none target organisms. It is also likely that those proteins have multiple receptors and different effects/modes of actions in vivo on humans and animals consuming cry proteins simultaneously, as it has been demonstrated by the paper of Bohn et al (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) <i>Daphnia magna</i> negatively affected by chronic exposure to purified Cry-toxins. <i>Food and Chemical Toxicology</i> 91: 130-140).</p>	<p>Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize 1507xMON 810xMIR162xNK603 grains is limited, and ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry and Vip proteins will not alter this conclusion.</p>

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Hungary	Ministry of Agriculture	II.1.3 Comparative analysis	According to Hungarian experts, the maize events 1507, MON810, MIR162 and NK603, all showed significant differences from their non-GM counterparts.	The GMO Panel assessed all the significant differences between maize 1507 x MON810 x MIR162 x NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the four-event stack maize and the non-GM comparator and falling under category III/IV were identified.
Hungary	Ministry of Agriculture	II.1.3.4 Comparative analysis of composition	<p>1.3.4 A significant difference between CHT 1507xMON810xMIR162xNK603 maize and control maize and between IHT 1507xMON810xMIR162xNK603 maize and control maize was observed for 40 of 71 analytes. Analytes expressing significant differences and/or lack of equivalences in the grain of IHT 1507xMON810xMIR162xNK603 maize are crude fat, isoleucine, leucine, and phenylalanine, vitamin B1 (thiamine), vitamin B5 (pantothenic acid) and γ-tocopherol, inositol.</p> <p>Analytes expressing significant differences and/or lack of equivalences in the grain of IHT 1507xMON810xMIR162xNK603 maize are γ-tocopherol, and crude fat (the differences in individual fatty acids are not discussed).</p> <p>Significant differences are significant, even if the values fell within the “range of natural biological variation observed in maize from outside study comparators, or intervals published in literature, including international reference values”. Significant differences are significantly different, and no evaluation of their “biological relevance of the observation” or “systematic examination of the data”, the “evaluation of patterns”, or “consideration of the biological meaning of the results” can change this. Every experiment is different, and so are the analytical methods used. The reason that scientific journals will not accept comparisons of data between different experiments is exactly this, since all circumstances, conditions in different experiments are different.</p>	The GMO Panel assessed all the significant differences between maize 1507 x MON810 x MIR162 x NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the four-event stack maize and the non-GM comparator and falling under category III/IV were identified.

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Hungary	Ministry of Agriculture	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	Plant height for CHT 1507xMON810xMIR162xNK603 maize was statistically different from the control maize.	Two independent sets of field trials were received: North America (2012) and North America (2015); both sets were considered, although for different purposes. Compositional data were provided only for the field trials in 2012. Agronomic-phenotypic data were measured in both sets, but they were assessed based only on data from the field trials in 2015, as those in 2012 did not include the measurement of yield components (yield and kernel weight) and therefore were considered incomplete. Suitability of materials and representativeness of the receiving environments were assessed for both years. Early stand count and yield for the four-event stack maize (treated) showed significant differences with respect to the non-GM comparator and fell into equivalence category III. Whether the differences can lead to an environmental adverse effect is considered in Section 3.4.4. of the scientific opinion.
Hungary	Ministry of Agriculture	II.1.3.7 Conclusion	It is stated in the Dossier that the compositional, the agronomic and phenotypic characteristics of 1507xMON810xMIR162xNK603 maize are comparable to those of the conventional counterpart and commercial reference maize lines, taking into account natural variation. It is not strictly so. The aim of these comparisons is to see unintended effects of the genetic modification and not to see if the new variety is, or is not in the range of conventional commercial reference maize lines. The statistical differences or lack of equivalences in the nutrient composition observed between 1507xMON810xMIR162xNK603 maize and the control line cannot be explained away by not having any biological relevance to the food and feed safety of 1507xMON810xMIR162xNK603 maize.	The GMO Panel takes note of the comment.

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Hungary	Ministry of Agriculture	II.1.4.1 Testing of newly expressed proteins	<p>According to Hungarian experts, the safety of the Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI or CP4 EPSPS proteins has not been proven previously, and the safety of all these proteins in one crop needs proper toxicological evaluation, considering the possible interaction between these transgenic proteins, as well as between the proteins and the herbicide(s) residues and metabolites used on them. The expression of multiple cry proteins has an additive effect on plant protection. It is also likely that those proteins have multiple receptors and different effects/modes of actions in vivo on humans and animals consuming cry proteins simultaneously, as it has been demonstrated by the paper of Bohn et al (2016). It was concluded in their paper that Cry-toxins in combination indicate alternative modes-of-action. The authors suggested that 'stacked events' may have stronger effects on non-target organisms, and that further studies are need to be done on the combinatorial effects of multiple Cry-toxins and herbicides that co-occur in the environment.</p> <p>It cannot be stated, since it was not proven, that the transgenic proteins expressed in 1507xMON810xMIR162xNK603 maize have a history of safe consumption as part of approved single GM events that are grown in the U.S. and other regions. 10 years or so cannot be called history, and these transgenic Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been a part of the food supply only for a few years. One cannot say that these transgenic proteins were consumed without incident and that they pose no significant risk of adverse toxic effects, since GM food in the US is not labelled, no one knows what amount of, and what type of the transgene(s) was consumed by whom and when. Based on these comments Hungarian experts suggest that there was an urgent need to perform animal feeding study(s) with rodents to assess reproductive, developmental and chronic toxicity, as well as food/feed safety of 1507xMON810xMIR162xNK603</p>	<p>Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize 1507xMON 810xMIR162xNK603 grains is limited, and ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry and Vip proteins will not alter this conclusion. The risk assessment of the herbicides and their metabolites is outside the remit of the GMO Panel.</p> <p>The GMO Panel takes note of the comment. (Please, refer to section 3.4.3.3 of the scientific opinion).</p>
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			<p>maize.</p> <p>It is stated that the intact transgenic proteins degrade in SIG and SIF, but this is no proof in vivo degradation. Protein degradation can be measured in vivo, but those measurements have not been performed.</p> <p>If a protein has a receptor(s) and/or a substrate(s), after binding to it/them it almost always becomes resistant to photolytic degradation. Cry proteins are likely to have such receptors, as well as transgenic enzymes have their substrates.</p> <p>The genes present in GM plants are different from the natural plant genes, since their sequence has been modified and/or are coupled with different regulatory elements. Our gut has never been exposed to such synthetic DNA sequencing before. Every cell is capable to take up sequences of RNA and DNA of differing length, so do microbes, although these sequences do not enter the germ cells.</p> <p>The CP4 EPSPS protein might not have synergistic or antagonistic effects with the other transgenes present in 1507xMON810xMIR162xNK603 maize, but the herbicide the CP4 EPSPS protein provides tolerance for does have an effect(s), and so has its residue(s) and metabolites on the gut flora.</p>	
Hungary	Ministry of Agriculture	II.1.4.4 Testing of the whole genetically modified food or feed	<p>There were problems with the 90 days feeding studies with the events 1507, MON810, MIR162 and with NK603. Based on the combined presence of the Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins, as well as the possible presence of glyphosate/glufosinate residues and metabolites, and based on comments in 1.3 and 1.4, Hungarian experts suggest that there is an urgent need to perform animal feeding study(s) with rodents to assess reproductive, developmental and chronic toxicity, as well as food/feed safety of 1507xMON810xMIR162xNK603 maize, before it can enter the food and feed chains.</p>	<p>Based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern in the composition of maize 1507 x MON810 x MIR162 x NK603 have been identified. Therefore, further animal studies on food/feed derived from the four-stack were considered not necessary</p>

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Hungary	Ministry of Agriculture	II.1.4.5 Conclusion of the toxicological assessment	Hungarian experts disagree with the conclusion that „1507xMON810xMIR162xNK603 maize and its sub-combinations are as safe for consumption as non-GM maize grain and no further testing is justified”.	The GMO Panel takes note of the comment.
Hungary	Ministry of Agriculture	II.1.5.1 Assessment of allergenicity of the newly expressed protein	<p>It is stated that Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been part of the food supply for years without any incident and there is no expectation that they pose a significant risk of adverse allergenic effects or otherwise pose a threat to food safety. As it was stated before, the transgenic Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been a part of the food supply only for a few years. One cannot say that these transgenic proteins were consumed without incident and that they pose no risks, since GM food in the US is not labelled, and in other countries as well as in the USA no one knows what amount and what type of transgene was consumed when and by whom.</p> <p>Cry proteins have allergic potentials, and can work as adjuvants (Adel-Patient et al., 2011; Guimaraes et al., 2008; Kroghsbo et al., 2008; Moreno-Fierros et al., 2003; Prasad and Shethna, 1975; Román Calderón et al., 2007; Vázquez- Padrón et al., 2000; Vázquez et al., 1999). Farm animals might be easily (and often) exposed to dust of 1507xMON810xMIR162xNK603 maize during feeding. Therefore, at least some evidence, such as screening with serums of patients with known and documented allergy are needed to prove no harm in addition to the in silico searches.</p>	The GMO Panel followed its guidance documents to assess the allergenic potential of maize 1507xMON810xMIR162xNK603 (EFSA GMO Panel, 2011; Regulation 503/2013). The conclusions of the assessment of allergenicity of the newly expressed proteins in the context of this application is described in section 3.4.3.4. The GMO Panel considers that there are no indications that the newly expressed proteins in maize 1507xMON810xMIR162xNK603 may be allergenic, in the context of this application.
Hungary	Ministry of Agriculture	II.1.5.2 Assessment of allergenicity of the whole genetically modified plant	Exposure through faeces of animals fed to the GM maize can have an effect on the environment and non-target organisms, and this was not considered in the risk assessment.	Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize 1507xMON 810xMIR162xNK603 grains is limited, and ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry and Vip proteins will not alter this conclusion.



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				The risk assessment of the herbicides and their metabolites is outside the remit of the GMO Panel.
Hungary	Ministry of Agriculture	II.1.6 Nutritional assessment	The monitoring plans for 1507xMON810xMIR162xNK603 maize is the same as for all other GM plants, including the same problems. No monitoring is carried out by independent observers and although the questioners are filled by operators, they are not available for inspection. Present methods used in Post Market Monitoring are not suitable to identify any risks. Even if any effects would be observed during monitoring, it would be impossible to tie those effects to any GM crops. Routine monitoring is conducted as a precaution and to detect unforeseen effects. The real question is, is there any effect which can be detected by general monitoring, when so many different GMO are in the feed and food supply? How can an effect, if found tied to any GM crop?	Monitoring is related to risk management, and thus a final adoption of a post-market monitoring (PMM) plan whenever needed falls outside the mandate of EFSA. For this particular application, as reflected in section 3.6.1. of the EFSA scientific opinion, the GMO Panel concluded that the four-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the non-GM reference varieties tested. Therefore, post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary
Hungary	Ministry of Agriculture	II.1.6.1 Nutritional assessment of the genetically modified food	The composition of 1507, MON810, MIR162, and NK603 GM maize events showed several significant differences between the GM maize event and their conventional counterpart.	The GMO Panel assessed all the significant differences between maize 1507 x MON810 x MIR162 x NK603 and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints showing significant differences between the four-event stack maize and the non-GM comparator and falling under category III/IV were identified.
Hungary	Ministry of Agriculture	II.1.6.3 Conclusion of the nutritional assessment	The composition of 1507, MON810, MIR162, and NK603 GM maize events showed several significant differences between the GM maize event and their conventional counterpart.	Based on the outcome of the comparative assessment (3.4.2.6.) the GMO Panel concluded that no nutritional assessment is needed since 1507 x MON810 x MIR162 x NK603 maize is nutritionally equivalent to the non-GM comparator and the non-GM reference varieties used.

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Hungary	Ministry of Agriculture	II.2 Exposure assessment — anticipated intake or extent of use	<p>The exposure assessment ignores the fact that a great many people are intolerant or allergic to wheat gluten and forced to switch and eat maize. Their number increases year by year. Instead of using wheat those persons use maize flour for cooking and baking. In addition, there are populations in Italy, Hungary, and Romania using maize flour and grit as staple food and eat more than does an average European person. Although not all maize consumed by livestock is the variety 1507xMON810xMIR162xNK603, but most GM maize events and stacks contain some Cry1F, PAT, Vip3Aa20, or PMI protein.</p>	Human dietary exposure to the different newly expressed proteins present in 1507xMON810xMIR162xNK603 maize was estimated considering a 100% replacement scenario that it is considered overly conservative when assessing potential risks linked to the intake of these proteins. Additionally, potential losses of the newly expressed proteins during processing are not considered, which also implies an overestimation of the current dietary exposure.
Hungary	Ministry of Agriculture	II.3 Risk characterisation	<p>The donor organisms from which genetic material was used for the creation of single events 1507, MON810, MIR162 and NK603 maize are <i>Bacillus thuringiensis</i> (cry1F, cry1Ab and Vip3Aa20 genes), <i>Streptomyces viridochromogenes</i> (pat gene), <i>Escherichia coli</i> (pmi gene), <i>Agrobacterium tumefaciens</i> (CP4 epsps gene), <i>Arabidopsis thaliana</i>, and Cauliflower Mosaic Virus. None of these organisms have ever been consumed as food or feed.</p> <p>Several significant differences were detected between 1507xMON810xMIR162xNK603 maize and its conventional counterpart (see 1.3 and 1.4) for compositional and agronomic characteristics.</p> <p>Therefore, 1507xMON810xMIR162xNK603 maize was not demonstrated to be nutritionally equivalent to grain produced from non-transgenic near-isogenic control maize.</p> <p>Hungarian experts do not support the conclusions that:</p> <p>a) Consumption of food and feed derived from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) is as safe as food and feed from conventional maize and no adverse effects on human or animal health are expected;</p> <p>b) Food derived from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) is comparable to food from conventional maize and is not nutritionally disadvantageous for the consumer</p>	The GMO Panel took note of the comment.

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			<p>compared to food which it is intended to replace;</p> <p>c) Food from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) does not mislead the consumer;</p> <p>d) Feed from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) does not harm or mislead the consumer by impairing the distinctive features of the animal products;</p> <p>e) Feed from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) is comparable to feed from conventional maize and is not nutritionally disadvantageous for animals or humans compared to the feed which it is intended to replace.</p> <p>The comparative assessment for the composition and agronomic characteristics indicated significant differences between the GM (IHT and CHT) and its comparator.</p>	
Hungary	Ministry of Agriculture	II.4 Post-market monitoring on the genetically modified food or feed	<p>Hungarian experts disagree with the conclusion that no post-market monitoring of GM food or feed products containing, consisting of, or derived from 1507xMON810xMIR162xNK603 maize (or any of its sub-combinations) is necessary.</p>	The GMO Panel took note of the comment.
Hungary	Ministry of Agriculture	II.6.2 Case Specific Monitoring (strategy, method and analysis)	<p>The authorisation request is for food and feed use only. Hungarian experts disagree with the evaluation of the characteristics of 1507xMON810xMIR162xNK603 maize in the ERA (Section 5 of Part II of this application) that the risk for potential adverse effects on human and animal health or the environment of this stacked GM maize is negligible.</p>	The GMO Panel took note of the comment.
Hungary	Ministry of Agriculture	II.6.3 General Surveillance (strategy, method)	<p>Hungarian experts would like to see case specific monitoring for following the health effects of 1507xMON810xMIR162xNK603 maize on humans and animals.</p>	<p>As the environmental risk assessment did not identify potential adverse environmental effects from the maize 1507 x MON 810 x MIR162 x NK603, no case-specific monitoring is required.</p> <p>For this particular application, as reflected in section 3.6.1. of the EFSA scientific opinion, the GMO Panel concluded that the four-event stack maize, as described in this application, does not raise any nutritional concern and is as safe as the non-GM comparator and the non-GM reference varieties tested. Therefore, post-market monitoring of food and feed from the four-event stack maize and</p>

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				its subcombinations, as described in this application, is not necessary.
Hungary	Ministry of Agriculture	II.6.4 Reporting the results of PMEM	General surveillance is the same for all GM plant events. The question is, if and when an effect found how can it be tied to any GM crop? 4.5 Unfortunately, in addition to existing networks (importers, traders, silo managers) no operators further down the food and feed chain have been selected for the general surveillance.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.
Hungary	Ministry of Agriculture	Part I – General information	General comments: Hungary has objected to the authorisation of 1507 maize, as well as MON810, the MIR162 and the NK603 GM maize varieties on strictly scientific basis. There is a moratorium on 1507 and MON810 varieties in Hungary. Since the stack1507xMON810xMIR162xNK603 GM maize variety contains both events Hungary has a moratorium, and the problems with the individual GM events are still valid, Hungary very strongly objects to authorisation of the stack 1507xMON810xMIR162xNK603 GM maize variety and other stacks containing the events 1507, MON810, MIR162 and NK603 GM.	The issues mentioned in the comment are outside the remit of the EFSA GMO Panel.
Italy	Ministry for Environment	II.1.2.2 Information relating to the genetically modified plant	On the 18th of december 2015 Pioneer Overseas Corporation, on behalf of Pioneer Hi-Bred International Inc., has presented an application for the authorisation of genetically modified plants and derived food and feed, not for cultivation, in accordance with Regulation (EC) No 1829/2003 of the genetically modified maize 1507xMON810xMIR162xNK603 and all sub-combinations with fewer of these events, independently of their origin (MON810xMIR162xNK603; 1507xMIR162xNK603; 1507xMON810xNK603; 1507xMON810xMIR162; 1507xMON810; 1507xMIR162; 1507xNK603; MON810xMIR162; NK603xMON810; and MIR162xNK603). This maize contains stacked events already authorized, furthermore the sub-combinations 1507xNK603 e NK603xMON810 have been authorized	

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		<p>with the Commission Decisions 2007/703/EC of 24 October 2007 e 2007/701/EC of 24 October 2007 respectively. As far as the sub combinations 1507xMON810xNK603, 1507xMON810 are currently being assessed in the framework of application EFSA-GMO-NL-2011-92 and the sub combination 1507xMIR162 in the framework of application EFSA-GMO-DE-2010-86, EFSA opinion within 47 days. We need to underline that some of the potential sub combination have not been evaluated and/or submitted to evaluation under European legislative framework.</p> <p>We think that it is necessary to give more information supporting the following statement (par. 1.2.2.2.): "As no interactions at the DNA and RNA level impacting on protein expression levels can be observed in the 1507xMON810xMIR162xNK603 maize compared to the single event lines and as there are no known mechanisms that could specifically impact on expression levels of any of the sub-combinations it can be reasonably assumed that no interactions impacting on expression levels are expected for the sub-combination". In fact, it is not clear how the applicant infers that all of the sub-combination do not show interactions, basing on the results of the comparison of the double-cross hybrid with only the single event lines.</p>	<p>The GMO Panel has assessed all single events and the higher stack. The information contained in the application was considered sufficient to conclude on the safety of the subcombinations of the events found in the four-event maize stack, in line with the IR 503/2013 and the EFSA GMO Panel subcombinations strategy document (Minutes of the GMO Panel 115<sup>th</sup> Plenary meeting, 17-18 May 2017, Annex 1, available at <a href="https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf">https://www.efsa.europa.eu/sites/default/files/event/170517-m.pdf</a>).</p>
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Italy	Ministry for Environment	II.6 Post-Market Environmental Monitoring Plan (PMEM)	<p>"Approach": the applicant refers only to substantial unintended losses of GM maize during loading/unloading of the viable commodities as a route for environmental exposure. Other routes of exposure of the environment (e.g. waste materials from processing or use of GM maize, transportation) were not assessed specifically. The applicant should analyze all potential routes of exposure, including waste material and transportation. Moreover, the notifier states that "Exposure can be controlled by clean up measures and the application of current practices used for the control of any adventitious maize plants, such as manual or mechanical removal and the application of herbicides (with the exception of glyphosate or glufosinate herbicides)". No clear responsibilities are assigned in case of accidental exposure, so it remains unclear who actually will be responsible for those clean-up measures: we ask to detail more this aspect. Lastly, according to the applicant, the operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable 1507xMON810xMIR162xNK603 maize: it is required to provide such guidelines to evaluate their effectiveness. "Existing systems": the applicant is working together with other members of the plant biotechnology industry within the European Association of Bioindustries (EuropaBio) and trade associations representing the relevant operators in order to implement an harmonised monitoring methodology. The links to COCERAL and UNISTOCK websites are non-correct: it is required to update these links. Moreover, not all European Member States are represented within these associations: therefore, it would be appropriate to provide explanations on the monitoring methodology adopted in the MS not represented. "Monitoring Methodology": the applicant states that the information collected will be evaluated and analyzed in order to assess the relevance: the method is not specified and then it is required to provide it. In the EFSA guidance on PMEM (EFSA Panel</p>	<p>Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.</p>
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			on Genetically Modified Organisms, 2011) is established that "In addition, applicants should provide raw data in order to allow different analyses and interrogation of the data and to allow scientific exchange and co-operation between applicants, Member States, the European Commission and EFSA": then, it would be appropriate that the applicant provides also the raw data, as well as the analyzes. Lastly, the notifier says that "Where information indicates the possibility of an unanticipated adverse effect, the authorisation holder will immediately investigate to determine and confirm whether a significant correlation between the effect and 1507xMON810xMIR162xNK603 can be established": we ask to specify the investigation method.	
Italy	Ministry for Environment	II.6.3 General Surveillance (strategy, method)	In the paragraph it is stated that "The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable 1507xMON810xMIR162xNK603 maize". In order to better evaluate the proposed general surveillance plan, it could be useful to know the content of the above-mentioned guidance because it is right during the handling of goods that unintended release into the environment can occur.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 1507 x MON 810 x MIR162 x NK603.
Italy	Ministry for Environment	II.6.4 Reporting the results of PMEM	As described by the EFSA guidance on PMEM (EFSA Panel on Genetically Modified Organisms, 2011), "GS plans should include questionnaires to those involved in the handling and processing of the GMP and its products and be designed to monitor whether unanticipated levels of loss, spillage and establishment are occurring and/or if there are any adverse environmental consequences". Nowhere in the PMEM proposed by the applicant were described questionnaires to the operators involved, nor how these questionnaires are structured, which information collect and how this information will be analyzed: it is required to provide this information.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA.

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Italy	Ministry for Environment	II.6.4 Reporting the results of PMEM	<p>it would be useful include in the annual monitoring report for the 1507xMON810xMIR162xNK603 maize information on foreseen amount of imported maize into the EU, ports, silos and processing facilities where the viable GM maize will be loaded/unloaded and transferred to, and transportation routes. In addition, it is advisable to specify in this paragraph if the annual report also contains the results of the screening of peer-reviewed publications conducted by the notifier (referred to in par. 6.4.5).</p> <p>Referenze/References:</p> <ul style="list-style-type: none"> <li>• EFSA Panel on Genetically Modified Organisms, 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879.</li> <li>• EFSA Panel on Genetically Modified Organisms, 2011. Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316.</li> <li>• EFSA Panel on Genetically Modified Organisms 2011. Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150.</li> </ul>	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA.
Netherlands	Dutch GMO Office	Part II – Scientific information	The Dutch CA has assessed the dossier with respect to the environmental and the food and feed safety of 1507xMON810xMIR162xNK603 maize and has no comments or requests for additional information in relation to the safety of this GM event.	The GMO Panel takes note of the comment.



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Norway	VKM	II.1.3.6 Effects of processing	<p>The conclusions drawn by the applicant that “there are no metabolic pathways affected or new metabolites produced in 1507xMON810xMIR162xNK603 maize” are not supported by the parameters provided using the targeted analyses described. Untargeted assays such as transcriptomics, proteomics and/or metabolomics are needed to support such statements. Although OECD and EFSA guidelines at present do not require such analyses, the conclusions, as they are currently worded, are misleading and the applicant should consider rephrasing or removing them.</p> <p>Furthermore, maize gluten meal (MGM) is a commonly used protein-rich ingredient in feeds for companion animals and fish. Processing steps to produce MGM are quite mild and the newly expressed proteins, such as Cry1F, Cry1Ab and Vip3Aa20, will possibly be present in MGM at considerably higher concentrations than in unprocessed maize. Especially Vip3Aa20 is present at relatively high levels in unprocessed maize grain (mg per kg level) and the Norwegian VKM GMO Panel considers that documentation regarding levels expected in MGM would be of value for considerations regarding hazard identification for untested non-target animals such as dogs and cats, as well as salmon, trout and other carnivorous farmed fish species.</p>	<p>The GMO Panel takes note of the comment.</p> <p>The GMO Panel takes note of the comment.</p>
Norway	VKM	II.1.4 Toxicology	<p>Based on data provided by the applicant, the GMO Panel is of the opinion that sufficient data are provided on the toxicological properties of the newly expressed proteins. No hazard indicating toxicity has been identified in any of the single event maize lines. However, information on synergistic/antagonistic interactions between the proteins in non-target organisms is lacking, especially at higher levels presumably present in processed maize products such as maize gluten meal.</p>	<p>The GMO Panel takes note of the comment. On the basis of the known biological function of the individual newly expressed proteins (Table 4), there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize 1507 × MON810 × MIR162 × NK603.</p>

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Norway	VKM	II.1.5 Allergenicity	<p><b>1.5 Allergenicity:</b>  The applicant claims that insecticidal proteins have not been identified as allergens or adjuvants. However, various studies indicate that effects due to Cry1Ac's adjuvant properties cannot be ruled out. Relevant levels of the insecticidal Bt proteins in processed maize products such as maize gluten meal should also be taken into consideration for allergenicity and adjuvant development in untested non-target animals.</p>	<p>The GMO Panel followed its guidance documents to assess the allergenic potential of maize 1507xMON810xMIR162xNK603 (EFSA GMO Panel, 2011; Regulation 503/2013). The conclusions of the assessment of allergenicity of the newly expressed proteins in the context of this application is described in section 3.4.3.4. The GMO Panel considers that there are no indications that the newly expressed proteins in maize 1507xMON810xMIR162xNK603 may be allergenic, in the context of this application. For additional information on adjuvanticity, please see additional references cited in the EFSA opinion referring to an EFSA document on adjuvanticity of Cry1Ac and an EFSA external report on adjuvanticity in general (EFSA, 2018b; Parenti et al., 2019).</p>
Norway	VKM	II.1.6 Nutritional assessment	<p>Data provided did not reveal performance differences between feeding groups in the broiler study conducted with maize 1507xMON810xMIR162xNK603 and its conventional counterpart and other commercial maize varieties. However, The VKM GMO Panel is of the opinion that data on residues levels of the intended herbicides glyphosate and glufosinate-ammonium should have been provided.</p>	<p>The GMO Panel takes note of the comment. The risk assessment of the herbicides and their metabolites is outside the remit of the GMO Panel.</p>
Sweden	National Food Agency	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	<p><b>COMMENT ON APPLICATION 127</b></p> <p>Although Appendix 1 of Annex 21 discuss the selection of field trial sites and maize materials grown in the field trials in general, the applicant could be asked for the pro and cons for including the site in Texas for field trials of maize based on the PHE4N x PHH9H genetic background.</p>	<p>The field trials were conducted in typical maize growing areas of North America, representing regions of diverse agronomic practices and environmental conditions, which is supported by the geographic map indicating the locations, the information provided on the variety of agronomic practice, soils and meteorological factors. In order to improve the representativeness of the selected field trials, EFSA published a guidance document on the agronomic and phenotypic characterisation of genetically modified plants (EFSA GMO Panel, 2015a). Application EFSA-GMO-NL-2015-127 was submitted during the transitional period of the GMO Panel guidance. Therefore, the requirements of the guidance document were not fully applicable for this application. Spontaneous information to integrate the selection of sites with new field trials were provided on 30/5/2016 and 06/06/2016. The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and management practices of the field trial sites are acceptable for receiving environments where the tested materials could be grown.</p>