**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| Austria | Federal Ministry of Health | A. Hazard identification and characterisation | **General remarks:**<br><br>The notifier used GM material from two different genetic backgrounds (PHW2Z and PHTFE). GM material derived from event 4114 bred into the first inbred line was used in the comparative assessment (Annex 8; Annex 9 ), while GM material derived from the other was used in feeding studies (e.g. Annex 20a ; Annex 23 ), a germination study (Annex 12 ) and an additional study evaluating expression levels of GM maize 4114 (Annex 10).<br><br>While for the evaluation of the stability of the expression respective data derived from different genetic backgrounds may provide an additional value, we would appreciate it if data presented for a particular event in an application dossier is derived from one breeding line only in order to ensure comparability and consistency among the data presented.<br><br>We request clarification regarding the use of the two different maize lines particularly with respect to the question which of them will be used for commercial development.<br><br>[Annex 8, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 9, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. and Canada Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 10, Expressed Trait Protein Concentration of a Maize Line Containing Events DP-ØØ4114-3, DAS-Ø15Ø7-1, DAS-59122-7, and Combined Trait Product DAS-Ø1507-1xDAS-59122-7: US and Canada Test Sites. Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 12, Evaluation of Germination and Dormancy of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Site. Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 20a, Thirteen-Week Rat Feeding Study with Maize Grain Containing Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 23, Thirteen-Week Rat Feeding Study with Maize Grain Containing Event DP-ØØ4114-3 (2). Dossier EFSA/GMO/NL/2014/123.] | The EFSA GMO Panel thanks Austria for this comment.<br><br>In the field trials conducted in US and Canada in 2011 and 2012 maize 4114 was introgressed into two different genetic backgrounds (PH705×PHW2Z and PH12SG×PHW2Z). In the field trials conducted in US in 2014 (additional trials submitted on 23/9/2015) maize was introgressed into PHR1J×PHW2Z. At each site/study, the non-GM comparator had a genetic background similar to the maize 4114 hybrid used, as documented by the pedigree. The GMO Panel considered the selected non-GM comparators to be suitable. Further details are provided in Section 3.3.1.1 of the scientific opinion.<br><br>The statistical analysis was in line with the requirements outlined in the EFSA guidance (EFSA GMO Panel, 2011). For the analysis, the data from the two different genetic backgrounds were pooled together both for the non-GM comparators and for maize 4114 soybean. Therefore, the statistical power of the tests was not affected. |
| Austria | Federal Ministry of Health | A, 2.1 Information relating to the genetic modification | **2.1.2 Nature and source of vector** used:<br><br>Scientific Information, p. 21: Please correct the sentence "The number of nucleotides in the plasmid pSB1 is 36,909 bp" to "Plasmid pSB1 contains 36,909 base pairs" or to "The number of nucleotides in the plasmid pSB1 is 73,818".<br><br>Scientific Information, p. 22: Please correct the first line according to the comment on page 21 (see above). | The GMO Panel takes note of this ambiguity in the text |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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|---|---|---|---|---|
| | | | **2.1.3 Source of donor nucleic acid(s) used for transformation, size, and intended function of each constituent fragment of the region intended for Insertion:**<br><br>Scientific Information, p. 27-28: The applicant states that "all constituents' fragments of the region intended for insertion have a history of safe use in food and feed."<br><br>Please be aware that "history of safe use" is a conflicting term without a clear scientific definition. It is doubtful whether it is possible to obtain valid conclusions on the risk of DNA fragments without a thoroughly designed accompanying post market monitoring programme.<br><br>Scientific Information, p. 29: The applicant states, "Although S. viridochromogenes has not been used as food source, it might be present in food unintentionally. "<br><br>It is not clear what the applicant wanted to explain with this statement. Anything may be present in food unintentionally - but this is no valid argument in favour of or against a potential hazard. | The GMO Panel takes note of these remarks |
| Austria | Federal Ministry of Health | A, 2.2.1 General description of the trait(s) and characteristics which have been introduced or modified | Scientific Information, p. 34: **The applicant states,** "Glufosinate-ammonium tolerance will allow growers to proactively manage weed populations." We would like to point the attention to the fact that the application of glufosinate is intended to be prohibited in the European Union from October 1st, 2017. From then on the respective herbicide tolerance trait does not add any grower or consumer benefits to the product. | The GMO Panel takes note of the comment made by Austria. |
| Austria | Federal Ministry of Health | A, 2.2.2 Information on the sequences actually inserted/deleted or altered | The data submitted for molecular characterisation of GM maize 4114 consist of Southern blots to demonstrate the presence of a single transgenic element containing the main functional elements of the transgenic construct. Appropriate controls were used and the pedigree history of test lines and the experimental setup is described well. The used probes cover all main functional transgenic elements of the insert as well as the entire backbone of the plasmid used for transformation.<br><br>However, the sensitivity of the Southern blot analyses was not assessed systematically, but is only assumed to be sufficient from results for detection of a positive control (i.e. the plasmid used for transformation) at 3 and 1 copies/genome equivalent. The notifier should submit an adequate assessment of the sensitivity of the analyses to detect any partial inserts in GM maize 4114.<br><br>**Specific comments:**<br><br>Scientific Information, p. 35: The applicant states, "Genomic DNA was extracted from leaves of 4114 plants." We would like to ask the EFSA GMO Panel to enquire the number of plants which have been analysed for Southern blotting.<br><br>Scientific Information, Fig. 1.2.10 (p. 39): The applicant states, "Hybridisation … with the | The GMO Panel considered the quality of the submitted data to be sufficient to conclude on the molecular characterisation of maize 4114.<br><br>Further information on the tested samples used for the Sourthen analysis is provided in Annex 4.<br><br>The GMO Panel acknowledges the limitations that may be associated with the southern analysis methodology (efficiency |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | ubiquitin promoter and intron probes resulted in a band of approximately 3100 bp and a band of greater than 8600 bp. "<br><br>On the blot four fragments are visible (lanes 1 and 2): An extremely faint band below a strong band at 4.8 kb, and two weaker bands at approx. 15 kb and 100 kb. We would like to ask the EFSA GMO Panel to ask the applicant how he has calculated the numbers for the 2 fragments to be of approx. 3100 bp and > 8600 bp. Both numbers fail to correspond to any appropriate fragment on the gel photos.<br><br>The Bcl I digest of PH1B5 (lane 5) shows a clear shift, the corresponding fragments in lanes 6 and 7 show double banding. Please explain the aberrations.<br><br>The applicant maintains, "The Bcl I-digested PHP27118 plasmid lanes did not produce the expected size bands, most likely due to the known sensitivity of Bcl I to Dam methylation." The approach of the applicant to apply a restriction enzyme with known deficiencies is questionable. The presented system is clearly inappropriate to determine fragment lengths.<br><br>We would like to ask the EFSA GMO Panel to enquire the reason for the double banding pattern of the 4.9 kb fragment in lanes 6 and 7. How can the applicant be sure that Bcl I cuts correctly plant genomic DNA if the enzyme is so sensitive to methylation?<br><br>Scientific Information, Fig. 1.2.11 (p. 40): Please explain the faint bands at 0.72, 1.7 and 3.0 kb.<br><br>Scientific Information, Fig. 1.2.15 and 1.2.16 (p. 44) (ORF25 terminator): Lane 7: Bands are shifted compared to lane 6. Please explain.<br><br>Ad Annex 4 (Southern Blot Analysis)<br><br>Ad Annex 4 (Southern Blot Analysis)<br><br>Figure 20 (p. 49)<br><br>Lane 1: The band of the positive control is too faint to provide evidence for a sufficiently sensitive Southern blot to exclude the presence of backbone fragments covered by Backbone probe 11 (please compare to Figures 22 and 24 where the positive controls are unambiguously clear).Figure 25 (p. 56) (Backbone probe 20 + 45)<br><br>Lane 1: The band of the positive control is too faint to provide sufficient evidence for a sensitive Southern blot to exclude the presence of backbone fragments. As the positive control fragments are too faint a band in the range of 6.1 kb in lanes 8 and 9 cannot be excluded decisively.<br><br>[Annex 4, Southern Blot Analysis of the F1*1 Generation of DP-ØØ4114-3 Maize to Verify Gene Copy Number and Integrity and Absence of Backbone DNA. Dossier EFSA/GMO/NL/2014/123.] | of restricton enzymes, gel electrophoresis step, band intensity etc.) however the provided data were considered sufficient to conclude on the molecular characterisation of maize 4114. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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|---|---|---|---|---|
| Austria | Federal Ministry of Health | A, 2.2.3 Information on the expression of the inserted/modified sequence | The notifier presents data for the concentrations of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in GM maize 4114 from plant material originating from field trials in the US (and one additional site in Canada in 2012) from two growing seasons (Annex 8 ; Annex 9 ). GM maize 4114 treated with conventional herbicides, GM maize treated with the intended herbicide (i.e. glufosinate) and a non-GM control also treated with conventional herbicides were grown at six (in 2011), respectively four (in 2012), different locations. However the assessment as well as the presentation of the data shows relevant deficiencies:<br><br>- The field trials for the comparative assessment in 2012 comprised 4 locations (Annex 9). However expression data were only used from 2 sites without providing a justification for this reduction in the data set.<br><br>- The statistical analysis is restricted to basic descriptive statistics, such as means and data ranges. No analysis of variance was conducted, neither within locations nor between locations to test for influences of the environment or the genetic background of the used hybrid lines.<br><br>The assessment of the variation of the expression of the inserts is important in order to assess whether the expected agricultural practice influences the range of expression of the transgenic proteins. This is also relevant to account for potential interactions with the respective environment (genotype x environment interactions). Thus, the notifier should include expression data from all available sites as requested by EFSA (EFSA 2010, EFSA pers. comm. 2014), thereby increasing the robustness of the data. Furthermore, the notifier should be requested to complement the exposure assessment with a detailed statistical analysis (i.e. an analysis of variance).<br><br>[Annex 8, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 9, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. and Canada Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>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.] | The GMO Panel acknowledges that protein expression data were collected from two out of the four sites used for compositional and agronomical data generation. However the provided information is in line with EFSA guidelines. |
| Austria | Federal Ministry of Health | A, 2.2.4 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant | The notifier concludes that the insert is stably integrated into GM maize 4114 from an assessment of plants from 5 generations of GM maize 4114 (F1*1, BC2F1*1, BC3F1*1, BC2F1*2, BC3F1*2) by means of PCR analysis (event specific as well as transgene specific) and phenotypic analysis for the transgenic herbicide resistance trait (Appendix 11). The pedigree history of the different GM maize 4114 generations assessed is adequately described. The data from individual PCR analyses are not provided.<br><br>We take note that the used methods cannot identify minor changes in the transgenic insert of GM maize 4114 upon propagation in the field in a comprehensive way and are therefore not | The provided information is considered adequate to conclude on the insert stability and Mendelan segregation.<br><br>The data provided is in line with the requirements of the GMO Panel (EFSA GMO Panel, 2011) and the Implementing |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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|---|---|---|---|---|
| | | | fully conclusive with regard to stability of the transgenic construct.<br><br>We do acknowledge that 100 individual plants from each generation were assessed for instabilities regarding the event as well as gene specific PCR-patterns and the functionality of the transgenic herbicide resistance trait, which is reasonable evidence to support the claim of the notifier that the insert is inherited stably and in a Mendelian way. However, the level of stability which can be detected by the experiments should be indicated.<br><br>**Specific comments**<br><br>No details on the exact location of the primers are given. In PHI-2009-107/700 and PHI-2009-107/701 the applicant refers to the study record of PHI-2009-015, in which the detailed procedures are documented. This report is not available. From the available documentation it is not clear whether the applicant used the same primers as for the data described in PHI-2009-107/700 and PHI-2009-107/701. This information would be important to evaluate the sequence coverage of the PCR results as described in Annex 11. The data shown to support the genetic stability may be based on only partial coverage of the insert, and thus the integrity of the insert is not sufficiently presented.<br><br>The Cry proteins inserted into 4114 maize confer resistance to lepidopteran and coleopteran pests, whereas the PAT protein confers tolerance to glufosinate ammonium. Accordingly, 4114 maize in total contains four active proteins conferring diverse traits, of which three are based on Cry proteins. To show the phenotypic stability the applicant describes the observed herbicide tolerance of the plants under investigation. These data match the observed segregation results. The phenotypic stability of the other three traits has not been investigated at all, although the insect resistances are an integral part of the 4114 maize.<br><br>According to the Commission Implementing Regulation (EU) No 503/2013 the applicant has to show, besides the genetic stability of the genetically modified plant, also the phenotypic stability of the introduced traits. In this context please also take notice of the "Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants" (EFSA Journal 2011) in which the following is stated "The applicant should also consider the safety implications of the loss of function of specific genes from multi-gene expression cassettes after their insertion into the plant." From the current dossier, it is not possible to evaluate whether the expression of the Cry proteins within the multi-gene expression cassette is stable over five generations.<br><br>It should be finally noted that, although Southern blot analysis does not facilitate assessing minor alterations to the integrity of the insert, the method used to analyse stability in the application at hands (Annex 11) is not satisfactory.<br><br>[Amended report from PHI-2009-107/700 and PHI-2009-107/701: Sequencing Characterization of Insert and Genomic Border Regions of Maize Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123<br><br>Annex 11, Segregation Analysis of Five Generations of a Maize Line Containing Event DP- | Regulation (EU) No 503/2013.<br><br>The GMO Panel considers there that is no indication of insert instability that would lead to the loss of any of the expression cassettes.<br><br>The provided data on the phenotypic stability of 4114 maize are in line with the relevant data requirments and were considered adequate by the GMO Panel.<br><br>The GMO Panel found no indication that would lead to the loss of any of the expression cassettes.<br><br>The GMO Panel considers that the data provided by the applicant is sufficient to conclude on the stability of the insert. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

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|---|---|---|---|---|
| | | | ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.] | |
| Austria | Federal Ministry of Health | A, 2.2 Information relating to the GM plant | 2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects:<br>Survivability (Scientific Information, p. 70f):<br>Although the survival and reproduction of maize is indeed limited under most European conditions, the applicant should acknowledge that maize seeds may survive and reemerge as volunteers in the following year. In this case the introduced traits would confer a selective advantage that could furthermore spread by cross pollination. | The GMO Panel considers it unlikely that the intended traits of maize 4114 will provide a selective advantage to maize plants, except when they are exposed to glufosinate-containing herbicides or infested by insect pests that are susceptible to the Cry1F, Cry34Ab1 or Cry35Ab1 proteins. However, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.<br>The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient. Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low. Even if cross-pollination would occur, the GMO Panel considered that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties. |
| Austria | Federal Ministry of Health | A, 2.3 Conclusions | 2.4 Conclusions:<br>In relation to the genetic stability it is noted that the phenotypic stability was only shown for one trait (herbicide tolerance). Hence, the argumentation of the notifier that "phenotypic stability of the conferred traits was demonstrated across five sexual generations, showing Mendelian inheritance of the insert sequences and intended traits " is not correct. | The provided data on the phenotypic stability of 4114 maize are in line with the relevant data requirments and were considered adequate by the GMO Panel. |
| Austria | Federal Ministry of Health | A, 3. Comparative assessment | The data presented for the comparative analysis were generated in field trials conducted in the US (6 sites in 2011, 3 sites in 2012) and Canada (1 site in 2012) (Annex 8; Annex 9 ). The assessment comprises compositional and agronomic data as well as data on the expression of the inserted trait.<br>In addition, a study evaluating germination and dormancy of GM maize 4114 seed was presented (Annex 12). However, it is unclear where the used seed material was produced, how it was produced, and whether the current requirements for field studies were observed for this study.<br>The applicant states that the field sites "reflect the different meteorological and agronomic parameters under which the product is expected to be grown" and refers to their "inclusion in the commercial maize growing regions of North America" as the basis for their selection | In addition to the field trials conducted in US and Canada in 2011 and 2012, respectively (Annex 8 and Annex 9), to assess the agronomic characteristics and nutrient composition of maize the applicant provided an additional agronomic study performed in US in 2014 at eight sites Study Number PHI-2014-035).<br>Field trials included data on monthly temperature, rainfall, and irrigation as well as on maintenance product applications. The field trials were conducted in major maize growing areas of the US and Canada, representing regions of diverse agronomic practices and environmental conditions. This was |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | (Scientific Information, p. 135). However, neither a rationale for the selection of the different test sites nor evidence for their representativeness for the agronomic regions, where maize is commercially grown, is presented. Furthermore, no characterisation of the test sites (e.g. soil type, information regarding the typical local agronomic practices or prevailing pest or disease pressure) beside basic weather data (e.g. monthly temperature, rainfall) and information on plant protection product applications is presented.<br><br>However, Implementing Regulation (EU) No 503/2013 (EC 2013) states that the choices for the selected field trial sites which are to reflect different meteorological and agronomic conditions shall be justified (see Chapter 1.3.2.1.b). Thus, we request that the applicant provides information on the above mentioned aspects and a clarification on the material used in the evaluation of germination and dormancy.<br><br>[Annex 8, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 9, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. and Canada Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 12, Evaluation of Germination and Dormancy of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Site. Dossier EFSA/GMO/NL/2014/123.<br><br>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.] | considered satisfactory by the GMO Panel.<br><br>The GMO Panel wants to add that guidance on the agronomic and phenotypic characterisation of GM plants was published on 24/6/2015 (EFSA GMO Panel, 2015). For all applications submitted 24 months or more after the publication date need to adhere to the requirements laid down in this guidance document are fully applicable. These requirements include a comprehensive and accurate description of various aspects of the receiving environments (such as geographical location, agrometeorological data, soil characteristics, cropping history, post-harvest conditions and crop management practices). |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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|---|---|---|---|---|
| Austria | Federal Ministry of Health | A, 3.1 Criteria for the selection of comparator(s) | The breeding diagram (Scientific Information, p. 20) shows a very complex pattern of crossing and selfing steps to produce the field test materials from the transformation event T0. For the comparative assessment, in the end, two different GM test materials were produced: F1*9 and F1*13.<br><br>The use of two different genetic backgrounds in the test material is justified by commercial interests (maize breeding programs). Anyhow, the use of multi-step breeding schemes does not favour the approach outlined in EFSA Guidance (EFSA 2010; EFSA 2011) that "a conventional counterpart with a genetic background that is as close as possible to the GM plant shall be selected ".<br><br>Particularly, the notifier should provide a convincing justification for producing test material by crosses with inbred lines (line PH581, line PHR03) which are not in the genetic background of the conventional counterparts (Scientific Information, p. 20). This multi-step breeding schemes could have an influence on the natural variation between the test material (F1*9 or F1*13) and the control material (PH705/PHW2Z or PH12SG/PHW2Z) used.<br><br>The notifier is requested to substantiate his line of argumentation that natural variation is not influenced by presenting relevant literature data.<br><br>[EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111.<br><br>EFSA, 2011. Guidance of the GMO Panel on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 9(5):2149: 1-21.] | Maize 4114 was introgressed via backcrossing into different inbred lines.<br><br>In the field trials conducted in US and Canada in 2011 and 2012 maize 4114 was introgressed into two different genetic backgrounds (PH705×PHW2Z and PH12SG×PHW2Z). In the field trials conducted in US in 2014 (additional trials submitted on 23/9/2015) maize was introgressed into PHR1J×PHW2Z. At each site/study, the non-GM comparator had a genetic background similar to the maize 4114 hybrid used, as documented by the pedigree. The GMO Panel considered the selected non-GM comparators to be suitable. |
| Austria | Federal Ministry of Health | A, 3.2 Field trials: experimental design and statistical analysis | Two different test materials were used in the 2011 and 2012 field tests. Mainly because of marketing reasons: Scientific Information, p. 73: "…to more closely represent commercial varieties entering the market."<br><br>By using two different test materials, and thus two comparisons (in one statistical model), an additional factor of uncertainty is introduced in the statistical analysis. From a statistical point of view, it is not the same whether to test two different test materials against two control lines at 10 sites overall, or to test one test material against one control line at 10 sites.<br><br>There is a natural variation between the test line F1*9 and the test line F1*13, and there is a | The statistical analysis was in line with the EFSA 2011 guidance document (EFSA GMO Panel, 2011). For the statistical analysis, the data from the two different genetic backgrounds were pooled together both for the non-GM comparators and for the GM maize. Therefore, the statistical power of the tests was not affected. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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|---|---|---|---|---|
| | | | natural variation between the control line PH705/PHW2Z and the control line PH12SG/PHW2Z. This should be accounted for:<br><br>EFSA Guidance (EFSA 2010, p. 7f) notes the importance that experiments are designed to have adequate probabilities of Type II errors, also termed "statistical power". Thus, it has to be ensured that the statistical design chosen by the applicant has at least the same statistical power as the design proposed by EFSA (as outlined in EFSA 2010, Chapter 2 "Proposals concerning field trial design").<br><br>The notifier is requested to address these points and provide answers.<br><br>In conclusion, it is recommended to provide two independent statistical analysis of the available raw data. One for year 2011 with test material F1*9 and one for year 2012 with test material F1*13. A comparison of the results with the data already submitted by the notifier should be made to verify and confirm the conclusions drawn by the notifier.<br><br>[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.] | |
| Austria | Federal Ministry of Health | A, 3.3 Compositional analysis | The compositional analysis was performed in Northern America in the years 2011 and 2012. All sites used a randomised complete block design and contained conventional herbicide-treated 4114 maize (CHT 4114 maize), intended herbicide-treated 4114 maize (IHT 4114 maize), and conventional herbicide-treated control maize (non-GM conventional counterpart), and commercial reference lines.<br><br>In the year 2011, field trials were conducted at six US field sites. The test substance was hybrid maize seed from the F1*9 generation in PH705 x PHW2Z genetic background. The control maize was non-GM hybrid maize seed (F1 seed) derived from the cross between lines PH705 andPHW2Z.<br><br>In the year 2012, field trials were conducted at four sites (three USA, one Canada). The test substance was hybrid maize seed from the F1*13 generation in PH12SG x PHW2Z genetic background. The control maize was non-GM hybrid maize seed (F1 seed) derived from cross between lines PH12SG and PHW2Z.<br><br>Ad Annex 13:<br><br>The CHT 4114 maize analysis shows statistical differences between the GMO and the control maize for 29 endpoints (Types 2 and 4, listed in Tables 12-13, page 58f).<br><br>The IHT 4114 maize analysis shows statistical differences between the GMO and the control maize for 35 endpoints (Types 2 and 4, listed in Tables 14-15, page 59f).<br><br>Similarities in the statistical differences exist between the CHT and the IHT analysis:<br><br>• Crude fat, phosphorus (forage maize) | The GMO Panel assessed all significant differences between maize 4114 and its non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. No endpoints showing significant differences between maize 4114 and its non-GM comparator and falling under category III/IV were identified. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
|---|---|---|---|---|
| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | • Moisture, crude protein, ash (proximates, grain)<br>• Stearic acid, oleic acid, linoleic acid, arachidic acid, eicosenoic acid, behenic acid (fatty acids, grain)<br>• Arginine, isoleucine, leucine, lysine, phenylalanine, serine, tyrosine (amino acids, grain)<br>• Total tocopherol, γ-tocopherol (vitamin, grain)<br>• phytic acid (secondary plant metabolites, grain)<br>These 21 endpoints are statistically different in all tested groups and all endpoints show the same direction of significant difference in both analysis: Thus, in both the CHT and IHT analysis:<br>• stearic acid is statistically lower in the GMO,<br>• oleic acid is statistically lower in the GMO,<br>• linoleic acid is statistically higher in the GMO,<br>• the seven amino acids are statistically higher in the GMO,<br>• etc.<br>This aspect of the compositional analysis need to be further addressed in the Scientific Information. The Scientific Information (p. 87ff) only provides a discussion (with many details such as % difference, range, boxplot, tolerance interval, etc.) of the following endpoints: phosphorus, sodium, vitamin B5, raffinose, trypsin inhibitor.<br>At least, the same analysis is requested for the 21 endpoints listed above, for which the null hypothesis of no difference must be rejected and the conclusion is that the GMO maize 4114 is different from its conventional counterpart regarding these endpoints.<br>Please also note that EFSA Guidance recommends, "Frequencies of significant results of the proof of difference tests over the complete set of considered endpoints should be reported and discussed" (EFSA 2010, p. 56).<br>[Annex 13, Statistical Analysis of Nutrient Composition Data of a Maize Line Containing Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.<br>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.] | |
| Austria | Federal Ministry of Health | A, 3.3 Compositional analysis | Ad Annex 13 (cont.):<br>Some of the endpoints even have additional aspects that need further assessment:<br>• The statistical difference for endpoint "moisture (%FW)" is highly significant (p value < | The GMO Panel assessed all significant differences between maize 4114 and its non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. No endpoints showing significant |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| | | | 0.0001) for both analysis (CHT and IHT). The mean of the control maize is 17.5, and the means of the GMO 4114 maizes are 18.8 and 18.9.<br><br>It should be discussed if these differences in moisture content can be explained e.g. by unaccurate handling of the samples after harvest (storage) or other circumstances.<br><br>• The "fatty acid" pattern between the control maize and the GM test maize is highly significantly different: Stearic acid is highly significantly lower (p-values = 0.000315 and 0.000279) in the GM 4114 maize. Oleic acid is highly significantly lower (p-values = 0.00734 and 0.00897) in the GM 4114 maize. But, linoleic acid is highly significantly higher (p-values = 0.00951 and 0.0274) in the GM 4114 maize. The long chain fatty acids arachidonic acid, eicosenoic acid are both highly significantly lower in the GM 4114 maize (with very low p-values from 0.000922 to < 0.0001).<br><br>It should be further addressed whether the significantly changed fatty acid pattern may indicate changes in other metabolic pathways or minor metabolites that are not part of the compositional analysis.<br><br>• High differences were also found for "γ-tocopherol", which is significantly lower in the GM 4114 maize (p-values = 0.000878 and < 0.0001). The result for "γ-tocopherol" obviously impacts the total tocopherol content, which is also highly significantly lower in the GM 4114 maize. The individual site analysis shows dissimilarities between the 10 sites and also within the sites (compare Table 11, p. 55f).<br><br>• Another highly significant difference was found for phytic acid (p-values = 0.00142 and 0.00238). The phytic acid content in the GM 4114 maize is significantly higher.<br><br>The notifier is requested to address the abovementioned points.<br><br>It is also requested to carry out a direct statistical comparison between the CHT 4114 maize data and the IHT 4114 maize data. A direct comparison is valueable for assessing of potential influences of the expected agricultural practice (i.e. glufosinate treatment) on the phenotypic and agronomic characteristics and the composition of GM maize 4114.<br><br>According to EFSA Guidance (EFSA 2010, p. 10), "applicants should allow for the possibility of checking for possible site-specific effects, i.e. genotype by site interactions. If genotype x site interactions are identified, then it is important that each individual site trial is sufficiently well-replicated to allow a credible site-specific analysis at each of the sites. Therefore the requirements for the levels of replication are based on power considerations for single field trials (per site)."<br><br>The notifier presents the results of a site-specific analysis including an individual-site analyses and an evaluation of genotype-by-environment (GxE) interaction, which is appreciated. The results of these two analyses are presented in Annex 13 , Table 10, page 43ff.<br><br>The notifier, unfortunately, presents these results without any accompanying text or/and further discussion (however, table footnotes (a, b) are given indicating significant differences | differences between maize 4114 and its non-GM comparator and falling under category III/IV were identified. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | or interactions observed).<br>The notifier is kindly requested to compare the results of the across-site analysis and the site-specific analysis. Particularly, the abovementioned 21 endpoints should be studied and reviewed in relation to site-specific effects.<br>[Annex 13, Statistical Analysis of Nutrient Composition Data of a Maize Line Containing Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.<br>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.] | |
| Austria | Federal Ministry of Health | A, 3.4 Agronomic and phenotypic characteristics | The agronomic and phenotypic characteristics were analysed conducting field trials in Northern America in the years 2011 and 2012. All sites used a randomised complete block design with 4 replications (blocks), which is appreciated.<br>All 10 sites contained conventional herbicide-treated 4114 maize (CHT 4114 maize), intended herbicide-treated 4114 maize (IHT 4114 maize), and conventional herbicide-treated control maize (non-GM conventional counterpart), and commercial reference lines.<br>In the Scientific Information (p. 93) it is said, "A total of 20 agronomic characteristics were evaluated. Comparative analyses (i.e. difference test and equivalence test) were performed for all 20 characteristics."<br>From the 20 characteristics, 8 belong to pollen evaluation (pollen viability-shape and colour at different times) and are not agronomic features in the strict sense, and so there remain only 12 endpoints for agronomically characterising of GM maize 4114.<br>Although the new EFSA guidance document on agronomic assessment of GM plants (EFSA 2015) has been published in June this year, this new standard may be seen as a template for an adequate agronomic evaluation of GM maize.<br>So, the agronomic characteristics lack essential endpoints such as yield and moisture. (Moisture, in fact, is provided in the compositional analysis.) Yield has to be considered an essential endpoint in agronomic assessment of GM maize. Seed weight should usually be also included in GMO assessment, but is missing in this notification.<br>Leaf diseases are not distinguished, but summarised under the term "Disease Incidence". Not even in Annex 8 and Annex 9 (each Table 12) there is information what kind of leaf diseases occurred at what field sites, especially as in 2012 greater damage was caused by leaf diseases. From a scientific point of view, the information on leaf diseases is completely insufficient.<br>There is also no information on what insect pests caused the "Insect Damage", even when the damage observed was at a low level. Again, for a scientific evidence, the information on insect damage is completely insufficient.<br>For both abovementioned characteristics, the submitted information is completely insufficient | The applicant submitted field trials conducted in US and Canada in 2011 and 2012 to assess the agronomic and phenotypic characteristics of maize 4114 an additional field trial performed in US in 2014 on eight sites was submitted by the applicant, following a request from the GMO Panel to include the endpoint "yield".<br>Fourteen and fifteen agronomic and phenotypic endpoints were analysed in total in the 2011/2012 and 2014 field trials, respectively:<br>Early population, seedling vigour, time to silking, time to pollen shed, pollen viability and colour, plant height, ear height, stay green, disease incidence, insect damage, stalk lodging, root lodging, final population, and yield (only in the 2014 field trials).<br>In addition to the field trials, seed characteristics of maize 4114 were also tested under controlled conditions. Seed germination of maize 4114 was compared with that of its non-GM comparator. Seeds were incubated under controlled conditions at three different temperature regimes and the numbers of germinated (normal and abnormal) and non-germinated (hard, imbibed and dead) seeds were counted.<br>The GMO Panel considered that the endpoints measured were sufficient to assess the agronomic and phenotypic characteristics of maize 4114. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | and cannot contribute to a proper risk assessment of GM maize 4114. Therefore, with regard to biotic interactions, the notifier is requested to provide detailed data in order to substantiate his conclusions that "the agronomic and phenotypic characteristics of 4114 maize, with the exception of the introduced traits, are comparable to those of the conventional counterpart and commercial reference maize lines, taking into account natural variation."<br><br>The Scientific Information gives a very brief discussion in Chapter 1.3.5 "Comparative Analysis Of Agronomic And Phenotypic Characteristics" which is only half a page. For allowing conclusions to be drawn on the safety of the GM maize 4114, it is required to provide a more detailed analysis and discussion which, at the least, addresses all type 2 characteristics (listed in Annex 16 , p. 30f) and compares the results of the CHT GM maize and the IHT GM maize analysis, since a number of endpoints show similar trends of significant differences (e.g. plant height, root lodging).<br><br>[Annex 8, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 9, Agronomic Characteristics, Expressed Trait Protein Concentration, and Nutrient Composition of a Maize Line Containing Event DP-ØØ4114-3: U.S. and Canada Test Sites (EU Study Format). Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 16, Statistical Analysis of Agronomic Characteristic Data of a Maize Line Containing Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.<br><br>EFSA, 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. The EFSA Journal 13(6):4128: 1-44.] | |
| Austria | Federal Ministry of Health | A, 3.4 Agronomic and phenotypic characteristics | Ad Annex 12<br><br>Evaluation of germination and dormancy in seed derived from GM maize 4114:<br><br>This present study, carried out to illustrate the performance of modified maize line 4114 concerning reproduction, dissemination and survivability, is a very short and poor version of a study.<br><br>Materials:<br><br>Maize line 4114 was tested with its non-modified, near-isoline maize variety; as reference substances only two non-modified varieties were chosen for the test.<br><br>The study gives no information about the origin of the seed: growing sites (identical growing site or different ones), growing conditions (e.g. whether conditions, soil), harvesting conditions, sampling, etc.<br><br>Methods - A. Experimental design: | The GMO Panel takes note of the comments made by Austria. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | It is said by the notifier, "No broken or damaged seed were included in any of the germination tests." This does not correspond to ISTA-rules, where in the description of "pure seed" a "half-seed"-definition is used and therefore damaged seed must not be removed. (ISTA is not part of the references anyway, which should be an essential part of each germination-study!)<br>Methods - B. Germination Tests:<br>• "Warm germination" is carried out for 10 days (ISTA: 04/07 days)<br>• "Cold germination test" (this is not an ISTA method)<br>• "Diurnal germination test" (this is not an ISTA method)<br>• ISTA does not lay down specifications for relative humidity<br>Results and discussion:<br>Due to the fact that the trial was carried out to compare line 4114 only with its non-modified near isoline and 2 reference varieties, just without replication of the trial, we cannot underline the statistical statements and the conclusion based on these results.<br>In conclusion, it would be preferable that the test designs on germination follow ISTA-rules (or AOSA).<br>[Annex 12, Evaluation of Germination and Dormancy of a Maize Line Containing Event DP-ØØ4114-3: U.S. Test Site. Dossier EFSA/GMO/NL/2014/123.] | |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
|---|---|---|---|---|
| Austria | Federal Ministry of Health | A, 4.2 Assessment of newly expressed proteins | It is well known that synergistic and additive effects both between Bt toxins and other compounds do occur. In general, synergistic effects can be characterised by findings that exceed those that can be predicted from those of the single components. These effects are under discussion as to whether they could be used commercially to enhance the toxicity of Bt toxins in pest insects. However, it is also known that in some cases toxicity in non-target organisms may be enhanced, causing unexpected risks for the environment and human health.<br><br>There are uncertainties about the effects of cry toxins on mammals and humans. Very few have been tested for their effects on humans. Some Cry proteins are cytotoxic to human or mouse cells, but surprisingly not to insects. Moreover, the toxicity was cell-type specific, meaning that if the wrong kind of cellular tissue culture is used in the assay, toxicity may be underestimated. Cry toxin proteins may also stimulate an immune response leading to the need to test them as allergens (Heinemann 2010).<br><br>[Heinemann JA, 2010. Potential human health risks from Bt plants. Biosafety Briefing, TWN Third World Network, January 2010, 1-10.] | Given that environmental exposure of non-target organisms to spilled maize 4114 grains or occasional feral GM maize plants arising from spilled GM grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the GM plant with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins will not alter this conclusion.<br><br>Allergenicity as well as adjuvanticity/immunogenicity of the Cry proteins expressed in maize 4114 were assessed by the EFSA GMO Panel. In the context of this application, no concerns on allergenicity were identified. The EFSA GMO Panel also noted that there is no information available on the structure or function of the newly expressed Cry1F, Cry34Ab1, and Cry35Ab1 proteins that would suggest an adjuvant effect, of the individual proteins or their simultaneous presence in maize 4114, resulting in or enhancing an eventual specific immunoglobulin E (IgE) response to a bystander protein. |
| Austria | Federal Ministry of Health | A, 4.5 Assessment of the whole food and/or feed derived from GM plants | The applicant presents results of a repeated-dose 90-day toxicity study with 4114 maize (Annex 20a). As one of the results, two male rats in the 4114 group, verum without glufosinate treatment - (animal nos 205 and 208), were diagnosed with bilateral, multiple renal tubule tumors (RTT) of the amphophilic-vacuolar (AV) type in association with multifocal atypical tubular hyperplasia. In animal no. 205, there were 4-5 benign adenomas in each kidney. In animal no. 208, there was one benign adenoma in each kidney in addition to a single carcinoma in one kidney. These results were discussed by a pathology working group. The group came to the conclusion that the proliferative renal tubule cell lesions present in two 4114 group males (animal nos 205 and 208) were spontaneous and not related to consumption of the 4114 diet. To ascertain this, another, this time specifically renal-focused repeated-dose 90-day oral toxicity study with 4114 maize was done (Annex 23 ). The applicant argues that in this test there was no histologic evidence of renal toxicity or preneoplastic proliferative lesions in the kidneys of any animals.<br><br>We would like to comment on the notifier's argumentation as follows:<br><br>Coming to the first test (Annex 20a) which showed tumors exclusively in the male verum group, besides the pathological and epidemiological (historical control) attempt to explain, it would be interesting what is the statistical probability of an incidence of 16.7 % (2 out of 12) against a background incidence (historical control) to be accidental vs. being statistically linked to the treatment (with confidence intervals). And with the second test (Annex 23) which | The GMO Panel has considered all the information provided by the applicant in support of the adenomas and carcinomas and renal tubule hyperplasias observed in two males fed test diet (4114), and was able to concluded these are spontaneous lesions unrelated to consumption of the test diet. Moreover, the overall macroscopic and microscopic examinations of selected organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test materials in the diet (section 3.4.3.4 of the opinion). |

| Country | Organization | Reference | Comment | GMO Panel response |
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| | | | should illuminate the seen effects in the first study the applicant argues, as already said before, that there was no histologic evidence of renal toxicity or preneoplastic proliferative lesions in the kidneys of any animals.<br><br>But having a closer look to the renal pathological data of the male verum group animals there is a substantial number of findings as against the control and which are at least partly not seen in the female verum group. A detailed explanation of these phenomena would be necessary.<br><br>Moreover, in none of the provided tests specific parameters tracing possible immunotoxicity and/or allergenicity - under the aspects mentioned above - have been investigated. Specific immune system (as for instance immunoglobuline) investigations should be done to reveal even weak immunomodulatory effects in the short term in the sense of the EFSA "Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment" (EFSA 2014).<br><br>And last but not least, as already said elsewhere, with the given study batteries and designs, no final evidence is possible with reference to long-term (especially appropriate for foodstuffs), reproductive or developmental effects. Increased attention has to be paid to even very slight deviations from control groups in different parameters because of the very small concentrations/dosages of the active principles, at least with whole GM food/feed, which can be used.<br><br>[Annex 20a, Thirteen-Week Rat Feeding Study with Maize Grain Containing Event DP-ØØ4114-3. Dossier EFSA/GMO/NL/2014/123.<br><br>Annex 23, Thirteen-Week Rat Feeding Study with Maize Grain Containing Event DP-ØØ4114-3 (2). Dossier EFSA/GMO/NL/2014/123.<br><br>EFSA, 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. The EFSA Journal 12(10):3871: 1-25.] | In the risk assessment of food and feed from GM plants, investigations of the possible effects on the immune function of consumers are mainly focused on the potential allergenicity evoked by the newly expressed proteins or by the whole plant and dedicated settings and approaches are in place for their evaluation (EFSA GMO Panel, 2011; Regulation (EU) 503/2013)).<br><br>The 90-day study defined by OECD TG 408 (OECD, 1998) includes the evaluation of the immune system by a number of routine clinical pathology and morphological tools (*e.g haematology parameters, such as white blood cell count and differential WBC count; clinical chemistry parameters, such as total serum protein or albumin; organ weight of spleen and thymus; histopathology of the spleen, lymph nodes, thymus, Peyer's patches and bone marrow*).<br><br>Based on the assessment of this study and the overall weight of evidence, no additional target studies on the immune system are considered needed. The same is valid for target studies reproductive or developmental effects. |
| Austria | Federal Ministry of Health | E. ERA | General remarks:<br><br>The EFSA Guidance on the ERA of GM plants lists specific areas of risk, each of which should be addressed in a stepwise manner during the ERA (cf. EFSA 2010). The first step is problem formulation (PF), "in which all important questions for the risk characterisation are to be identified" (EFSA 2010, p.14). This step is crucial for the ERA as it enables to focus the ERA process and to determine what to assess and at what level of detail. Therefore, the PF should lead to formulation of clearly phrased, testable risk hypotheses.<br><br>However, for all of the risk areas the applicant provided negatively formulated risk hypotheses, i.e. in the form of "…should not result in harm/in adverse effects…". This seems to be in | The GMO Panel takes note of the comment raised by Austria.<br><br>Considering the scope of application EFSA-GMO-NL-2014-123, which excludes cultivation, the ERA of maize 4114 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and 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 4114 grains during transportation and/or |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | contradiction to the general risk assessment approach outlined in the EFSA guidance documents (e.g. EFSA 2010).<br><br>In these guidance documents "equivalence testing" was introduced in order to decrease the potential for statistical outcomes, which indicate that no hazard exists, where indeed there is one. This approach was additionally implemented in order to implement a test design with sufficient statistical power to appropriately detect effects (see Perry et al. 2009). In "equivalence testing" - applied in the comparative assessment – the null hypothesis is one of inequality (in contrast to "difference testing", where the null hypothesis is one of equality). When an analogous approach is applied to ERA, the null hypothesis must be one of 'effect' instead of 'non-effect'. Only if tests are designed with sufficient statistical power to actually detect effects, can the results be related to the limits of concern (LoC), which should indicate the extent of ecological effects, which is deemed biologically significant and of sufficient magnitude to cause harm. However, based on the risk hypothesis formulated by the notifier no adequate test designs can be developed.<br><br>Additionally, the notifier does not follow the stepwise procedure outlined by EFSA for the ERA. For instance regarding the risk area of "potential interactions with non-target organisms" the notifier fails to conduct a proper exposure assessment and already concludes on the risk in the course of problem formulation. However, non-target organisms in soil and water may be exposed to GM maize 4114 material through accidental spillage or the expressed Bt toxins via faeces produced by animal fed GM maize 4114. Thus, the notifier should submit data for the characterisation of the potential exposure instead of merely inferring it from existing degradation data.<br><br>These inconsistencies and misconceptions in the ERA approach lead to insufficient data presentation underlying the ERA and complicate the assessment of the ERA by authorities. Therefore, we request that the applicant performs an appropriate problem formulation, including all the relevant aspects identified by EFSA (EFSA 2010, p.16).<br><br>[EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111.<br><br>Perry JN, Ter Braak CJ, Dixon PM, Duan JJ, Hails RS, Huesken A, Lavielle M, Marvier M, Scardi M, Schmidt K, Tothmeresz B, Schaarschmidt F, van der Voet H, 2009. Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. Environ Biosafety Res 8(2): 65-78.] | processing (EFSA GMO Panel, 2010).<br><br>The GMO Panel considers that environmental exposure of non-target organisms to spilled maize 4114 grains or occasional feral GM maize plants arising from spilled GM grains is limited. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
|---|---|---|---|---|
| Austria | Federal Ministry of Health | E, 3.2.1 Step 1: Problem formulation | **5.3.2.1. Step 1: Problem formulation**<br><br>Page 142: The applicant maintains, "The uptake of plant genes by micro-organisms, although a rare event, may hypothetically occur." We would like to point to the fact that the transfer of plant genes to bacteria has been demonstrated in situ (Bertolla et al. 2000) and in evolutionary terms (Smith et al. 1992). Although plant-to-bacteria gene transfer may be a rare event, it is no "hypothetic" process. Please delete "hypothetically".<br><br>The applicant maintains that "horizontal gene transfer of non-mobile DNA fragments between unrelated organisms (such as plants to micro-organisms) is extremely unlikely to occur under natural conditions." We would like to point to the fact that all inserted transgenes (cry1F, cry34Ab1, cry35Ab1, and pat ) are of prokaryotic origin and, thus, per se have the potential to recombine with similar counterparts in appropriate bacterial acceptor strains. Several members of the genus Bacillus have been shown to be capable to develop competence under naturally occurring conditions (de Vries and Wackernagel 2004; Johnsborg et al. 2007; Johnston et al. 2014).<br><br>The applicant maintains that there is a "lack of efficient mechanisms of integration of unrelated chromosomal DNA." This statement is misleading as the applicant generates the impression that chromosomal plant DNA is completely incompatible to bacterial DNA blinding out that the transgenes are of bacterial origin, and thus, in principle, prone to recombination with homologous or similar DNA sequences in competent bacteria.<br><br>The applicant mentions a "low-level temporal persistence of gene-sized plant DNA fragments." We would like to point to the fact that transgenic DNA is continually released via pollen, root exsudates and during decay of plant material (de Vries et al. 2003). Transgenic DNA is detectable in soils up to 2 years (Gebhard and Smalla 1999), accumulative effects may be expected in natural habitats (de Vries et al. 2003; de Vries and Wackernagel 2004). Exposure of soil bacterial populations by plant-derived transgenic DNA occurs over vast arrays of crop growing areas probably for decades. The indigenous flora of the gastrointestinal tract of animals is affected by a life-long exposure with transgenic DNA via feed. We would like the EFSA GMO Panel to take into account that a low-level temporal persistence may in fact be astoundingly long lasting.<br><br>[Bertolla F, Pepin R, Passelegue-Robe E, Paget E, Simkin A, Nesme X, Simonet P, 2000. Plant genome complexity may be a factor limiting in situ the transfer of transgenic plant genes to the phytopathogen Ralstonia solanacearum. Appl Environ Microbiol 66(9): 4161-4167.<br><br>de Vries J, Heine M, Harms K, Wackernagel W, 2003. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an Acinetobacter sp. Appl Environ Microbiol 69(8): 4455-4462.<br><br>de Vries J, Wackernagel W, 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant Soil 266(1-2): 91-104.<br><br>Gebhard F, Smalla K, 1999. Monitoring field releases of genetically modified sugar beets for | The GMO Panel takes note of the comment raised by Austria. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol Ecol 28(3): 261-272.<br><br>Johnsborg O, Eldholm V, Havarstein LS, 2007. Natural genetic transformation: prevalence, mechanisms and function. Res Microbiol 158(10): 767-778.<br><br>Johnston C, Martin B, Fichant G, Polard P, Claverys JP, 2014. Bacterial transformation: distribution, shared mechanisms and divergent control. Nat Rev Microbiol 12(3): 181-196.<br><br>Smith MW, Feng DF, Doolittle RF, 1992. Evolution by acquisition: the case for horizontal gene transfers. Trends Biochem Sci 17(12): 489-493.] | |
| Austria | Federal Ministry of Health | E, 3.2.1 Step 1: Problem formulation | 5.3.2.1. Step 1: Problem formulation (cont.):<br><br>Scientific Information, p. 143, third paragraph: The applicant describes the prerequisites necessary for homologous recombination, but avoids mentioning different mechanisms for DNA integration like homology-facilitated illegitimate recombination, which relies on short anchor sequences (e.g. 153 bp) and very short stretches of microhomology (3 - 10 bp) (de Vries and Wackernagel 2004) for the integration of foreign DNA.<br><br>Scientific Information, p. 143, fourth paragraph: The applicant proposes a "lack of competence of most bacteria to take up foreign DNA." We would like to indicate that it is likely that all gamma-Proteobacteria are carriers of competence gene homologs (Cameron and Redfield 2006). Natural genetic transformation is widely distributed among different taxonomic bacterial groups (Claverys and Martin 2003). It is to be assumed that many more bacterial species than identified by now can develop competence under naturally occurring conditions (Johnston et al. 2014). The environmental conditions necessary to induce competence have not been identified, yet, for the bulk of bacteria, but this does not mean that they are a priori not capable to take up foreign DNA under natural conditions (Seitz and Blokesch 2013).<br><br>We would like to point to the fact that the applicant is establishing preconditions not empirically verified.<br><br>Scientific Information, p. 143, fifth paragraph: The applicant maintains that "the gene (i.e. cry1F, cry34Ab1, cry35Ab1, and pat) is not under the control of a promoter which has activity in prokaryotic organisms" in support of his final conclusion in the problem formulation step. This is not correct. The pat gene is under the control of the CaMV promoter, which has been shown to be active in bacteria (Assaad and Signer 1990). In contrast to the statement of the applicant, a CaMV-pat gene fragment, if successfully transferred, would be functional in a prokaryotic environment.<br><br>[Assaad FF, Signer ER, 1990. Cauliflower mosaic virus P35S promoter activity in Escherichia coli. Mol Gen Genet 223(3): 517-520.<br><br>Cameron AD, Redfield RJ, 2006. Non-canonical CRP sites control competence regulons in | The GMO Panel takes note of the comment raised by Austria.<br><br>The GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genetic elements from maize 4114 to bacteria does not raise any environmental safety concern (see Section 3.5.1.2 of the Scientific Opinion). |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | Escherichia coli and many other gamma-proteobacteria. Nucleic Acids Res 34(20): 6001-6014.<br>Claverys JP, Martin B, 2003. Bacterial "competence" genes: signatures of active transformation, or only remnants? Trends Microbiol 11(4): 161-165.<br>de Vries J, Wackernagel W, 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant Soil 266(1-2): 91-104.<br>Johnston C, Martin B, Fichant G, Polard P, Claverys JP, 2014. Bacterial transformation: distribution, shared mechanisms and divergent control. Nat ev Microbiol 12(3): 181-196.<br>Seitz P, Blokesch M, 2013. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. FEMS Microbiol Rev 37(3): 336-363.] | |
| Austria | Federal Ministry of Health | E, 3.2.3 Step 3: Exposure characterisation | Scientific Information, p. 147: The applicant discusses the direct exposure of micro-organisms to glufosinate ammonium in the digestive tract of animals. In our opinion the exposure of bacteria to transgenic plant DNA is to be analysed in this section. The applicant should provide quantitative information on the copy number of transgenes which will be released in soil or the animal gastrointestinal tract. As these data are missing the exposure characterisation is in fact missing.<br>We would like to ask the EFSA GMO panel to ask the applicant for the relevant data to obtain a reasonable exposure characterisation. | The GMO Panel takes note of the comment raised by Austria. |
| Austria | Federal Ministry of Health | E, 3.2.6: Step 6: Conclusions | As the applicant bases his line of argumentation on several assumptions (e.g. lack of efficient mechanisms of integration of unrelated chromosomal DNA, low-level temporal persistence of gene-sized plant DNA fragments, lack of competence of most bacteria to take up foreign DNA, disregarding illegitimate homologous recombination mechanisms and mosaic gene formation) not substantiated by actual empirical evidence and on misconceptions (e.g. inactivity of CaMV promoter in bacteria) as well as on an inadequate (i.e. actually missing) exposure characterisation, the conclusions are biased and not sufficiently well-founded.<br>We would like to ask the EFSA GMO Panel to insist at least on a proper exposure assessment. | The GMO Panel takes note of the comment raised by Austria. |
| Austria | Federal Ministry of Health | E, 4. Post-Market Environmental Monitoring (PMEM) | 4.1 General:<br>In the monitoring plan at hands the notifier refers to the respective requirements contained in the Guidance Document of the EFSA GMO Panel published in 2006. This Guidance, however, has been revised since then, in particular by the current EFSA Guidance Document on PMEM (EFSA 2011a), and the requirements as regards structure and content of notifications were further elaborated (c.f. Implementing Regulation No. 503/2013). The notifier should review the submitted monitoring plan to establish that all requirements according to current guidance and | 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.<br>The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 4114. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | regulation have been taken into account appropriately. Among others the following specific issues need to be addressed during revision by the notifier according to the current requirements:<br><br>The notifier does not specifically consider potential exposure of EU environments to GM maize 4114 other than by unintended release of the GM maize e.g. via substantial losses during loading or unloading for processing into animal feed or human food products. Other exposure scenarios should be considered according to current EFSA guidance (EFSA 2011a), e.g. accidental spillage during transport, comingling 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 4114.<br><br>The notifier, furthermore, does not present a plan for monitoring the environmental exposure by GM maize 4114 using appropriate methods (i.e. standardised methodologies for sampling and identification of GM maize 4114).<br><br>Since the ERA presented for GM maize 4114 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 4114, the fate of transgenic materials in the environment and potential environmental impacts should be addressed by CSM (compare Züghart et al. 2011).<br><br>The general recommendations by EFSA from the evaluation of previous monitoring of other GM crops (EFSA 2011b; EFSA 2012) should be considered by the notifier and the respective suggestions should be implemented, e.g. as regards the literature review, etc…<br><br>[EFSA, 2011a. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316: 1-40.<br><br>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.<br><br>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.<br><br>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.] | As the environmental risk assessment (ERA) did not identify potential adverse environmental effects from the maize 4114, no case-specific monitoring is required.<br><br>Considering the scope of the application EFSA-GMO-NL-2014-123, interactions of occasional feral maize 4114 plants with the biotic and abiotic environment are not considered to be relevant issues by the GMO Panel. The analysis of HGT from maize 4114 to bacteria did not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the comparative analysis, the routes and levels of exposure, the GMO Panel concluded that maize 4114 would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.<br><br>Considering the scope of application EFSA-GMO-NL-2014-123, which excludes cultivation, the ERA of maize 4114 mainly took 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 4114 grains during transportation and processing (EFSA GMO Panel, 2010). |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| Austria | Federal Ministry of Health | E, 4.3. General Surveillance (approach, method and analysis) | As noted in the general comment 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 2011).<br><br>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.<br><br>The notifier only states that the responsibilities for the General Surveillance of GM maize 4114 are shared between the authorisation holder and third parties, such as operators involved in the import, handling and processing of viable GM maize 4114 (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.<br><br>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.<br><br>The notifier should therefore indicate the national organisations which will be involved in each individual EU Member State and not only the associations at EU level. It must be clear before placing on the market of GM maize 4114 which existing networks will be involved to which degree.<br><br>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 4114 in food or feed must be taken into account according to Regulation (EC) 1829/2003 (Art. 5.5b and Art.17.5b). Therefore e.g. respective medical or veterinary networks should be involved for the surveillance of unanticipated effects on human and animal health.<br><br>The methodology of the proposed General Surveillance is based on passively collecting information. A proactive approach of GS, including specific activities for monitoring of accidental spillage and the potential establishment of GM maize 4114 in the environment, should also be be proposed and implemented by the notifier (see general remarks to this Notification).<br><br>The notifier states that the surveillance based on the 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 4114. The general reference to HACCP principles as included in the monitoring plan thus needs to be better specified by the notifer. | The GMO Panel takes note of the comments made by Austria.<br><br>The GMO Panel considered that the scope of the post-market environmental monitoring (PMEM) plan provided by the applicant is consistent with the intended uses of maize 4114.<br><br>Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | In conclusion, the proposed monitoring plan is considered inappropriate for addressing relevant issues of PMEM of GM maize and thus cannot be regarded as sufficiently elaborated for the monitoring of potential environmental exposure by GM maize 4114.<br>[EFSA, 2011. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316: 1-40.] | |
| Austria | Federal Ministry of Health | A. Hazard identification and characterisation | Detection method:<br>The presented method describes the quantitative detection of GM maize 4114. The detection method uses TaqMan technology and event specific primers, i.e. one primer resides within the transformed insert and one in the plant genome.<br>During validation procedure criteria according to the ENGL requirements (http://gmocrl.jrc.ec.europa.eu/guidancedocs.htm) were measured as e.g. specificity, robustness, R2 value, PCR efficiency, dynamic range, trueness, precision, repeatability, reproducibility, quantitation limit (LOQ), detection limit (LOD).<br>The results meet the ENGL requirements for GMO validation.<br>The detection method for GM maize 4114 was sent for validation to CRL. The current evaluation status of the method is "Step 3 (experimental testing) ongoing" (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx). | Not in the EFSA GMO Panel remit. |
| Belgium | Belgian Biosafety Advisory Council | A, 3.3 Compositional analysis | As in previous submissions, data of crude fibre, acid detergent fibre and neutral detergent fibre are presented; this approach is relevant for animal feed; data of dietary fibre, soluble and insoluble dietary fibre are more relevant in human nutrition. | The GMO Panel takes note of the comment made by Belgium. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| France | Ministère de l'Economie (Consommation) | A, 2.2 Information relating to the GM plant | A.2. Caractérisation moléculaire<br><br>A.2.2. Informations concernant la plante génétiquement modifiée<br><br>L'analyse moléculaire du maïs 4114 a été réalisée par Southern blot et séquençage des fragments de jonction, en 5' et 3' de l'insert, entre l'ADN-T et l'ADN génomique de la plante. Ces analyses ont été réalisées sur les générations F1*1 (croisement des plantes de la génération T1 avec la lignée PH1B5) et T3, respectivement. Les comparateurs utilisés étaient, à juste titre, les lignées conventionnelles PHWWE et PH1B5 pour les Southern blots et PHWWE pour les analyses de séquences.<br><br>**ENGLISH TRANSLATION**<br><br>A.2. Molecular characterisation<br><br>A.2.2. Information related to the genetically modified plant<br><br>The molecular analysis of corn 4114 was performed by Southern blot and sequencing of the junction fragments, at 5' and 3' of the insert, between the T-DNA and the genomic DNA of the plant. These analyses were performed on generations F1*1 (crossing plants from generation T1 with the line PH1B5) and T3, respectively. The comparators used were, rightly, conventional lines PHWWE and PH1B5 for the Southern blots and PHWWE for the sequence analyses. | The GMO Panel thanks France for the assessment. |
| France | Ministère de l'Economie (Consommation) | A, 2.3 Conclusions | A.2.3. Conclusions de la caractérisation moléculaire<br><br>Les éléments présentés dans le dossier relatifs à la caractérisation moléculaire du maïs génétiquement modifié 4114 ne soulèvent pas de questions particulières liées à la consommation de ce maïs.<br><br>**ENGLISH TRANSLATION**<br><br>A.2.3. Conclusions of the molecular characterisation<br><br>The evidence presented in the dossier which relates to the molecular characterisation of the genetically modified corn 4114 does not raise any particular issues associated with the consumption of this corn. | The GMO Panel thanks France for the assessment. |
| France | Ministère de l'Economie (Consommation) | A, 3.1 Criteria for the selection of comparator(s) | A.3 Evaluation comparative<br><br>A.3.1 Choix de l'équivalent non transgénique et des comparateurs supplémentaires<br><br>Deux essais ont été réalisés, l'un en 2011 et le second en 2012. Dans chaque essai, le maïs 4114 est comparé avec une variété témoin et 12 variétés commerciales conventionnelles. Dans l'essai de 2011, l'évaluation porte sur la génération F1*9 du maïs 4114 et la variété témoin est l'hybride PH705 x PHW2Z. Dans l'essai de 2012, l'évaluation porte sur la génération F1*13 du | Maize 4114 was introgressed via backcrossing into different inbred lines.<br><br>In the field trials conducted in US and Canada in 2011 and 2012 maize 4114 was introgressed into two different genetic backgrounds (PH705×PHW2Z and PH12SG×PHW2Z). In the field trials conducted in US in 2014 (additional trials submitted on 23/9/2015) maize was introgressed into PHR1J×PHW2Z. |

| Country | Organization | Reference | Comment | GMO Panel response |
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| | | | maïs 4114 et la variété témoin est l'hybride PH12SG x PHW2Z. Les variétés témoins partagent plus de 97 % d'identité génétique avec les générations F1*9 et F1*13 du maïs 4114. Par ailleurs, les maïs commerciaux utilisés en 2012 sont différents de ceux utilisés en 2011 pour tenir compte de l'arrivée de nouvelles variétés sur le marché et de leur adaptation aux sites expérimentaux (synchronisation des maturités).<br><br>**ENGLISH TRANSLATION**<br><br>A.3 Comparative assessment<br><br>A.3.1 Choice of non-transgenic equivalent and additional comparators<br><br>Two tests were performed, the first in 2011 and the second in 2012. In each test, corn 4114 is compared with a control variety and 12 conventional commercial varieties. In the 2011 test, the assessment focuses on generation F1*9 of corn 4114 and the control variety is hybrid PH705 x PHW2Z. In the 2012 test, the assessment focuses on generation F1*13 of corn 4114 and the control variety is hybrid PH12SG x PHW2Z. The control varieties share more than 97% genetic identity with generations F1*9 and F1*13 of corn 4114. Furthermore, the conventional corns used in 2012 are different from those used in 2011 to take into account the arrival of new varieties on the market and their adaptation to the experimental sites (synchronisation of maturities). | At each site/study, the non-GM comparator had a genetic background similar to the maize 4114 hybrid used, as documented by the pedigree. The GMO Panel considered the selected non-GM comparators to be suitable. |
| France | Ministère de l'Economie (Consommation) | A, 3.2 Field trials: experimental design and statistical analysis | A.3.2. Dispositif expérimental et analyse statistique des données issues des essais au champ pour l'analyse comparative<br><br>Pour l'analyse de composition chimique des grains et du fourrage, ainsi que l'analyse des caractéristiques agronomiques et phénotypiques, le maïs 4114, les variétés témoins (PH705 x PHW2Z en 2011 et PH12SG x PHW2Z en 2012) et les 12 variétés commerciales (3 variétés par site) ont été cultivés sur 6 sites aux USA en 2011 et sur 4 sites (3 aux USA et 1 au Canada) en 2012. Ces sites, localisés dans des zones de culture du maïs, présentent un large panel de conditions pédoclimatiques et de pratiques agronomiques. Le maïs 4114 a été cultivé avec ou sans traitement herbicide avec du glufosinate-ammonium (respectivement T et NT). Chaque modalité (variété témoin, variétés commerciales et variété génétiquement modifiée T et NT) 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 de l'EFSA (2011).<br><br>Les caractéristiques agronomiques, phénotypiques et de composition sont comparées à l'aide d'analyses de variance en regroupant les résultats de tous les sites expérimentaux. Une ANOVA est réalisée avec un modèle linéaire mixte incluant :<br><br>- un effet fixe "génotype" (indiquant s'il s'agit du maïs 4114 NT ou T, de la variété témoin ou des variétés commerciales),<br><br>- des effets aléatoires : "site", "bloc dans le site" et "variété commerciale".<br><br>Le modèle statistique utilisé, qui inclut un effet fixe "génotype" et un effet aléatoire "variété | The field trials were conducted in major maize growing areas of the US and Canada, representing regions of diverse agronomic practices and environmental conditions. At each site, the following materials were grown in a randomised complete block design with four replicates: maize 4114 not treated with the intended herbicide (maize 4114/not-treated), maize 4114 treated with glufosinate (maize 4114/treated), a non-GM comparator, and several commercial non-GM maize reference varieties (i.e. three in the 2011/2012 study and four in the 2014 study). All materials were treated (sprayed) with required maintenance pesticides (including conventional herbicides) according to local requirements. In total, 12 and 20 non-GM maize reference varieties were included across the field trial sites performed in 2011/2012 and 2014, respectively.<br><br>Maize 4114 was introgressed via backcrossing into different inbred lines.<br><br>In the field trials conducted in US and Canada in 2011 and 2012 maize 4114 was introgressed into two different genetic backgrounds (PH705×PHW2Z and PH12SG×PHW2Z). In the field trials conducted in US in 2014 (additional trials submitted |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | commerciale", correspond à celui proposé par l'EFSA (2011).<br>**ENGLISH TRANSLATION**<br>A.3.2. Experimental set-up and statistical analysis of the data derived from the field trials for the comparative analysis<br>For the analysis of chemical composition of the grains and feed, as well as the analysis of agronomic and phenotypic characteristics, corn 4114, the control varieties (PH705 x PHW2Z in 2011 and PH12SG x PHW2Z in 2012) and the 12 commercial varieties (3 varieties per site) were grown at 6 sites in the USA in 2011 and at 4 sites (3 in the USA and 1 in Canada) in 2012. These sites, which were located in corn-growing areas, exhibit a broad range of soils, climates and agronomical practices. Corn 4114 was grown with or without herbicidal treatment with glufosinate-ammonium (T and NT, respectively). Each arrangement (control variety, commercial varieties and genetically modified variety T and NT) was repeated four times at each site in accordance with an experimental plan of random blocks. The characteristics of that experimental plan comply with the recommendations of the EFSA (2011).<br>The agronomic, phenotypic and compositional characteristics were compared using analysis of variance, with the results from all of the experimental sites being combined. An ANOVA is carried out with a mixed linear model including:<br>- a 'genotype' fixed effect (indicating that this is corn 4114 NT or T, the control variety, or the commercial varieties),<br>- random effects: 'site', 'block within the site' and 'commercial variety'.<br>The statistical model used, which includes a 'genotype' fixed effect and a random 'commercial variety' effect, corresponds to that proposed by the EFSA (2011). | on 23/9/2015) maize was introgressed into PHR1J×PHW2Z. At each site/study, the non-GM comparator had a genetic background similar to the maize 4114 hybrid used, as documented by the pedigree. The GMO Panel considered the selected non-GM comparators to be suitable.<br>The statistical analysis of agronomic, phenotypic and compositional data from the field trials followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010, 2011). |
| France | Ministère de l'Economie (Consommation) | A, 3.2 Field trials: experimental design and statistical analysis | II.1.3.3. Sélection du matériel et des composés pour analyse<br>L'analyse de composition porte sur le grain cru et le fourrage (analyse réalisée aux stades R6 (maturité) et R4 (stade pâteux), respectivement). Le pétitionnaire ne fait pas référence au document consensus de l'OCDE (2002)* pour le choix des composés analysés, mais les analyses réalisées sont recevables. Par ailleurs, aucune donnée n'est fournie sur les produits dérivés du maïs 4114.<br>Tous les résultats sont exprimés par rapport au produit sec. Le maïs 4114 NT et T est comparé avec les variétés témoins et les 12 variétés commerciales, pour lesquelles il aurait été souhaitable d'avoir des informations sur leur représentativité de l'ensemble des variétés cultivées.<br>* OECD. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea Mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds No. 6. Organization of Economic Cooperation | Maize 4114 grains and forage harvested from the field trials in the North America in 2011/2012 were analysed for 84 constituents (9 in forage and 75 in grain). The analysis included the key constituents recommended by OECD (OECD, 2002). |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | and Development (OECD), Paris (France), 2002.<br><br>**ENGLISH TRANSLATION**<br>II.1.3.3. Selection of the material and compounds for analysis<br>The analysis of composition focuses on the raw grain and the feed (analysis performed at stages R6 (maturity) and R4 (dough stage), respectively). The petitioner does not refer to the OECD consensus document (2002)* for the choice of compounds analysed, but the analyses performed are admissible. Furthermore, no data is provided regarding the products derived from corn 4114.<br>All of the results are expressed relative to the dry product. Corn 4114 NT and T is compared with the control varieties and the 12 commercial varieties for which it would have been desirable to have information regarding how representative they are of the entirety of the varieties cultivated.<br>* OECD. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea Mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Series on the Safety of Novel Foods and Feeds No 6. Organization of Economic Cooperation and Development (OECD), Paris (France), 2002. | |
| France | Ministère de l'Economie (Consommation) | A, 3.3 Compositional analysis | A.3.3. Analyse comparative de la composition<br>Les mesures de 71 composés (62 pour les grains et 9 pour le fourrage) parmi les 84 analysés sont utilisables pour les analyses statistiques. Les résultats des tests statistiques ont été interprétés selon l'approche décrite par l'EFSA (2010)* , en classant les variables en 7 types (1 à 7) selon les résultats des tests de différence et 4 catégories (I à IV) après combinaison avec les résultats des tests d'équivalence. Il n'est pas possible de conclure pour les teneurs de trois composés dans les grains : sodium, vitamine B5 et inhibiteur de la trypsine. Sur la base des résultats obtenus pour les autres composés, l'analyse combinée de l'ensemble des sites d'expérimentation de 2011 et 2012 montre que le maïs 4114 (grains et fourrage) est équivalent aux variétés commerciales.<br>*Statistical considerations for the safety evaluation of GMOs, The EFSA Journal 2010; 8(1):1250.<br><br>**ENGLISH TRANSLATION**<br>A.3.3. Comparative analysis of composition<br>The measurements of 71 compounds (62 for grain and 9 for feed) out of the 84 analysed can be used for the statistical analyses. The results of the statistical tests were interpreted in | As mentioned by France, for three compounds in grains, sodium, vitamin B5 and trypsin inhibitor, the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties. Further, the GMO Panel assessed all significant differences between maize 4114 and its non-GM comparator, taking into account potential impact on plant metabolism and the natural variability observed for the set of non-GM commercial reference varieties. No endpoints showing significant differences between maize 4114 and its non-GM comparator and falling under category III/IV were identified. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | accordance with the approach described by the EFSA (2010)*, with the variables being classified in 7 types (1 to 7) depending on the results of the difference test and in 4 types (I to IV) after being combined with the results of the equivalence tests. It is not possible to reach a conclusion for the contents of three compounds in the grains: sodium, vitamin B5 and trypsin inhibitor. Based on the results obtained for the other compounds, the combined analysis of all the experimentation sites from 2011 and 2012 show that corn 4114 (grains and feed) is equivalent to the commercial varieties.<br><br>*Statistical considerations for the safety evaluation of GMOs, The EFSA Journal 2010; 8(1):1250. | |
| France | Ministère de l'Economie (Consommation) | A, 3.4 Agronomic and phenotypic characteristics | A.3.4. Analyse comparative des caractéristiques agronomiques et phénotypiques<br><br>Les caractéristiques agronomiques et phénotypiques ont été évaluées sur 20 paramètres. De même que pour l'analyse de composition, les résultats des tests statistiques ont été interprétés selon l'approche décrite par l'EFSA (2010), en classant les variables en 7 types (1 à 7) selon les résultats des tests de différence et 4 catégories (I à IV) après combinaison avec les résultats des tests d'équivalence. Il n'a pas été possible de conclure pour le paramètre "vigueur des plantules". Les résultats obtenus sur les autres paramètres montrent que le maïs 4114 est équivalent aux variétés commerciales.<br><br>**ENGLISH TRANSLATION**<br><br>A.3.4. Comparative analysis of agronomic and phenotypic characteristics<br><br>The agronomic and phenotypic characteristics were assessed on 20 parameters. As with the analysis of composition, the results of the statistical tests were interpreted in accordance with the approach described by the EFSA (2010), with the variables being classified in 7 types (1 to 7) depending on the results of the difference tests and in 4 types (I to IV) after being combined with the results of the equivalence tests. It was not possible to reach a conclusion for the parameter 'seedling vigour'. The results obtained in the other parameters show that corn 4114 is equivalent to the commercial varieties. | The GMO Panel concluded that none of the agronomic and phenotypic differences identified with respect to the non-GM comparator and the non-GM commercial reference varieties need further assessment regarding food and feed safety and its environmental impact. |
| France | Ministère de l'Economie (Consommation) | A, 3.5 Effects of processing | A.3.5. Effets de la transformation<br><br>Le pétitionnaire affirme que les produits issus du maïs 4114 ne devraient pas être différents de ceux issus de maïs conventionnels mais ne présente aucune analyse des produits transformés.<br><br>**ENGLISH TRANSLATION**<br><br>A.3.5. Effects of processing<br><br>The petitioner states that the products derived from corn 4114 should not be different from those derived from conventional corn, but does not present any analysis of the processed products. | Based on the outcome of the comparative assessment, processing of maize 4114 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| France | Ministère de l'Economie (Consommation) | A, 3.6 Conclusions | A.3.6. Conclusions de l'évaluation comparative<br><br>La caractérisation phénotypique et agronomique et l'analyse de composition du maïs 4114 montrent que ce maïs est équivalent aux variétés conventionnelles pour les grains et le fourrage. Aucune analyse n'a été réalisée sur les produits issus du maïs 4114.<br><br>**ENGLISH TRANSLATION**<br><br>A.3.6. Conclusions of the comparative assessment<br><br>The phenotypic and agronomic characterisation and analysis of composition of corn 4114 shows that this corn is equivalent to the conventional varieties for grain and feed. No analysis was carried out in relation to the products derived from corn 4114. | Based on the outcome of the comparative assessment, processing of maize 4114 into food and feed products is not expected to result in products different from those of commercial non-GM maize varieties. |
| France | Ministère de l'Economie (Consommation) | A, 4.5 Assessment of the whole food and/or feed derived from GM plants | A.4. Toxicologie<br><br>A.4.5. Analyse de l'aliment (denrée alimentaire ou aliment pour animaux) génétiquement modifié entier<br><br>Le pétitionnaire fournit deux études :<br><br>- une étude initiale de toxicité sub-chronique de 90 jours chez le rat, réalisée en 2011 selon un protocole s'appuyant sur la ligne directrice OCDE 408 *1 et celle du Bureau de la prévention, des pesticides et des substances toxiques (OPPTS),<br><br>- une étude de toxicité sub-chronique de 90 jours chez le rat focalisée sur la fonction rénale, réalisée en 2013.<br><br>Dans les deux études, les farines de grains de maïs ont été incorporées dans les régimes alimentaires à la dose de 32 % uniquement. Les analyses réalisées sur les lots de grains utilisés ont porté sur leur composition, ce qui a permis de préparer des régimes équilibrés sur le plan nutritionnel, ainsi que sur les mycotoxines, les composés anti-nutritionnels et les résidus de pesticides.<br><br>L'étude initiale de 2011 a été réalisée avec six groupes de 12 rats mâles et 12 rats femelles, lignée Sprague Dawley, nourris avec des régimes alimentaires contenant une variété témoin, la variété génétiquement modifiée 4114, NT et T, et 3 variétés commerciales de référence. Le pétitionnaire précise que la variété témoin partage plus de 97 % d'identité génétique avec le maïs 4114 utilisé dans l'étude, mais il ne donne pas son identité exacte.<br><br>Cette étude, mise en œuvre avant les recommandations de l'EFSA (2011) *2 , ne comporte pas d'évaluation de la puissance des tests statistiques. Les animaux ont été observés conformément à la ligne directrice OCDE 408. Le pétitionnaire a réalisé des tests d'égalité des moyennes entre groupes avec des tests de Dunnett. L'erreur de type 1 a été fixée à 5 %. Les analyses ont été réalisées séparément pour les mâles et les femelles. Le pétitionnaire n'a pas utilisé de modèles mixtes prenant en compte les corrélations entre mesures répétées dans le | The GMO Panel takes note of the comments made by France.<br><br>The appropriateness of GM line and of the selected comparator used in the 90-day feeding study and the appropriateness of the statistical design were considered by the GMO Panel. |

| Country | Organization | Reference | Comment | GMO Panel response |
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| | | | temps sur un même animal pour les variables poids et consommation. Par ailleurs, les données brutes sous format électronique et les programmes de calcul ne sont pas fournis.<br><br>*1 OCDE (1998). Guideline for testing of chemicals N°408. Repeated dose 90-day oral toxicity study in rodents. Paris, France.<br><br>* 2 EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. The EFSA Journal 2011; 9(12):2438.<br><br>**ENGLISH TRANSLATION**<br><br>A.4. Toxicology<br><br>A.4.5. Testing of the whole genetically modified food or feed<br><br>The petitioner provided two studies:<br><br>- an initial 90-day sub-chronic toxicity study in rats, performed in 2011 according to a protocol based on OECD guideline 408*1 and that of the Office of Prevention, Pesticides, and Toxic Substances (OPPTS),<br>- a 90-day sub-chronic toxicity study in rats focused on renal function, performed in 2013.<br><br>In both studies, the cornmeals were incorporated into diets at a dose of only 32%. The analyses performed on the lots of grains used focused on their composition, which enabled preparation of nutritionally balanced diets, as well as mycotoxins, anti-nutritional compounds and pesticide residues.<br><br>An initial study in 2011 was performed with six groups of 12 male rats and 12 female rats, Sprague Dawley line, fed with diets containing a control variety, the genetically modified 4114, NT and T, and 3 commercial varieties as reference. The petitioner states that the control variety shares more than 97% genetic identity with the corn 4114 used in the study, but it does not give its exact identity.<br><br>This study, implemented prior to the EFSA recommendations (2011) *2, does not include an assessment of the power of the statistical tests. The animals were not observed in accordance with OECD guideline 408. The petitioner performed tests of equality of means between groups with Dunnett's tests. The type 1 error was fixed at 5%. The analyses were performed separately for males and females. The petitioner did not use mixed models taking into account the correlations between repeated measurements over time on the same animal for the variables weight and consumption. Furthermore, the raw data in electronic format and the computer programmes are not provided.<br><br>*1 OECD (1998). Guideline for testing of chemicals No 408. Repeated dose 90-day oral toxicity | |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | study in rodents. Paris, France.<br>* 2 EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. The EFSA Journal 2011; 9(12):2438. | |
| France | Ministère de l'Economie (Consommation) | A, 4.6 Conclusions | A.4.6. Conclusions de l'évaluation toxicologique<br>L'évaluation de la sécurité des protéines Cry1F, Cry34Ab1, Cry35Ab1 et PAT exprimées dans le maïs 4114 ne met pas en évidence d'éléments permettant de conclure que ces protéines ont un effet toxique sur la santé humaine et animale. Les études de toxicité sub-chronique de 90 jours chez le rat, réalisées avec des farines de grains de maïs 4114 traité ou non avec du glufosinate-ammonium, ne mettent pas en évidence d'effets ayant une signification biologique.<br>**ENGLISH TRANSLATION**<br>A.4.6. Conclusions of the toxicological assessment<br>The assessment of the safety of proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in corn 4114 does not show anything which would permit the conclusion that these proteins have a toxic effect on human and animal health. The 90-day sub-chronic toxicity studies in rats, performed with cornmeal from corn 4114 treated or not treated with glufosinate-ammonium, do not show any effects with a biological significance. | The GMO Panel thanks France for the assessment. |
| France | Ministère de l'Economie (Consommation) | A, 5.1 Assessment of allergenicity of the newly expressed protein | A.5. Allergénicité<br>A.5.1. Évaluation de l'allergénicité de la (des) protéine(s) nouvellement exprimée(s)<br>Les photocopies des résultats (pages 16 à 18) de la référence ID : GH-C 5367 sont de mauvaise qualité et ne permettent pas à l'évaluateur de vérifier précisément les résultats. Par ailleurs, la première page de la référence Glatt (1999) est illisible (problème à l'ouverture du fichier PDF).<br>**ENGLISH TRANSLATION**<br>A.5. Allergenicity<br>A.5.1. Assessment of allergenicity of the newly expressed protein(s)<br>The photocopies of the results (pages 16 to 18) from the ID: GH-C 5367 reference are of poor quality and do not allow the assessor to precisely check the results. Furthermore, the first page of the ID DuPont-3365 reference is illegible (problem opening the PDF file). | The Compentent Authority makes reference to studies related to the Cry1F and PAT proteins that have been previously assessed by the EFSA GMO Panel. For the assessment of the newly expressed proteins in maize 4114, the applicant bases its evaluation on previous studies performed in the context of applications involving the events 1507 and 59122 as single or stack events. In addition, Cry1F and PAT proteins are also present in many other events evaluated by EFSA. In all these assessments concerning Cry1F and PAT protein, no indications of safety concern were identified by the EFSA GMO Panel. Furthermore, the GMO Panel is not aware of any new information that would change these conclusions.<br>In the context of this applicaton on maize 4114, the applicant did not provide new experimental studies for the toxicity and allergenicity assessment of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT newly expressed proteins.<br>This Competent Authority considers that the allergenic potential of proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in maize 4114 may be considered negligible (please see the additional comment from the authority |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | | below). |
| France | Ministère de l'Economie (Consommation) | A, 5.4 Conclusions | A.5.4. Conclusions de l'évaluation de l'allergénicité<br>Sur la base des données et des commentaires fournis par le pétitionnaire, le potentiel allergénique des protéines Cry1F, Cry34Ab1, Cry35Ab1 et PAT exprimées dans le maïs 4114 peut être considéré comme négligeable. Par ailleurs, ces protéines n'ont apparemment pas de propriétés adjuvantes. Enfin, l'allergénicité du maïs 4114 reste identique à celle du maïs conventionnel.<br>**ENGLISH TRANSLATION**<br>A.5.4. Conclusions of the allergenicity assessment<br>Based on the data and comments provided by the petitioner, the allergenic potential of proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in corn 4114 may be considered negligible. Furthermore, these proteins apparently do not have any adjuvant properties. Finally, the allergenicity of corn 4114 remains identical to that of conventional corn. | The GMO Panel thanks France for the assessment. |
| France | Ministère de l'Economie (Consommation) | A, 6. Nutritional assessment | A.6. Evaluation nutritionnelle<br>Aucun effet significatif n'est observé. Par conséquent, pour le poulet de type standard en croissance, le maïs 4114 a les mêmes qualités nutritionnelles que le maïs témoin et les variétés de maïs conventionnelles testées dans cette étude.<br>**ENGLISH TRANSLATION**<br>A.6. Nutritional assessment<br>No significant effect was observed. Therefore, for the standard growing chicken, corn 4114 has the same nutritional qualities as the control corn and conventional corn varieties tested in this study. | The GMO Panel thanks France for the assessment. |
| France | Ministère de l'Economie (Consommation) | C. Risk characterisation | C. Caractérisation des risques<br>En se basant sur les consommations aiguës les plus élevées, la marge d'exposition (MOE) la plus faible est de 9643 pour la protéine Cry34Ab1 pour la classe d'âge « autres enfants » en Suède. En se basant sur les consommations chroniques les plus élevées, les marges d'exposition, calculées sur la base de l'étude de toxicité orale de 28 j (protéines Cry34Ab1 et Cry35Ab1), sont supérieures à 10000. Cette approche ne prend pas en compte les résultats des études de toxicité par administration répétée des farines de grains de maïs 4114 pendant 90 jours chez le rat. | The 90-day feeding study with repeated administration of cornmeal from corn 4114 in rats cannot be used to characterise risks associated to long-term consumption of the Cry34Ab1 and Cry35Ab1 proteins as the studies are carried out with the whole food/feed from corn 4114. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | **ENGLISH TRANSLATION**<br>C. Risk characterisation<br>Based on the highest acute consumption, the lowest margin of exposure (MOE) is 9643 for protein Cry34Ab1 for the age group 'other children' in Sweden. Based on the highest chronic consumption, the margins of exposure, calculated based on the 28-day oral toxicity study (proteins Cry34Ab1 and Cry35Ab1) are greater than 10 000. This approach does not take into account the results of the toxicity studies involving the repeated administration of cornmeal from corn 4114 over 90 days in rats. | |
| France | Ministère de l'Economie (Consommation) | A. Hazard identification and characterisation | Conclusions du Groupe de travail « Biotechnologie » de l'ANSES.<br>Les éléments présentés dans le dossier relatifs à la caractérisation moléculaire du maïs génétiquement modifié 4114 ne soulèvent pas de questions particulières liées à la consommation de ce maïs.<br>La caractérisation phénotypique et agronomique et l'analyse de composition de ce maïs montrent qu'il est équivalent aux variétés conventionnelles pour les grains et le fourrage.<br>L'évaluation de la sécurité des protéines Cry1F, Cry34Ab1, Cry35Ab1 et PAT exprimées dans le maïs 4114 ne met pas en évidence d'éléments permettant de conclure que ces protéines ont un effet toxique sur la santé humaine et animale. Les deux études de toxicité sub-chronique de 90 jours chez le rat ne mettent pas en évidence d'effets ayant une signification biologique.<br>Enfin, sur la base des éléments fournis dans le dossier, le potentiel allergénique des produits dérivés de ce maïs paraît extrêmement faible.<br>L'ensemble de ces éléments ne permet pas d'identifier un risque sanitaire lié à la consommation de grains et de produits dérivés du maïs 4114.<br>**ENGLISH TRANSLATION**<br>Conclusions of the ANSES 'Biotechnology' working group.<br>The evidence presented in the dossier which relates to the molecular characterisation of the genetically modified corn 4114 does not raise any particular issues associated with the consumption of this corn.<br>The phenotypic and agronomic characterisation and analysis of composition of the corn shows that it is equivalent to the conventional varieties for grain and feed.<br>The assessment of the safety of proteins Cry1F, Cry34Ab1, Cry35Ab1 and PAT expressed in corn 4114 does not show anything which would permit the conclusion that these proteins have a toxic effect on human and animal health. The two 90-day sub-chronic toxicity studies in rats do not show any effects with a biological significance.<br>Finally, based on the evidence provided in the dossier, the allergenic potential of the products | The GMO Panel thanks France for the assessment. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | derived from this corn appears to be extremely low.<br>None of these items allow a health risk associated with the consumption of grain and of products derived from corn 4114 to be identified. | |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A. Hazard identification and characterisation | The scope of application EFSA-GMO-NL-2014-123 covers import and processing of maize 4114 including all feed and food products containing, consisting of, or produced from the genetically modified maize 4114. Cultivation is not covered by this application.<br>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 4114 is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended use. Nevertheless, completion and/or clarification on some points of the dossier are recommended. | The GMO Panel takes note of the comments made by Germany. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A, 2.2 Information relating to the GM plant | Sequence information on flanking regions at each insertion site<br>Bioinformatic analyses were performed to characterize the location and nature of the T-DNA insertion in the maize genome. Additionally, the flanking genomic sequences were assessed for any potential gene disruption by the T-DNA insertion. The BLASTN search of the 5' flanking region against the EST database as well as the BLASTX search of this region against the protein database resulted in significant matches to a hypothetical glutaredoxin-like sequence. As a precaution, the expression of the corresponding mRNA in maize 4114 was assessed by Northern blot analysis. In this regard, it should be noted that more sensitive methods (e.g. qRT-PCR) are currently available to analyse mRNA expression of a putative gene. Generally, state-of-the-art methods should be used in current applications to work on such questions.<br>Open Reading Frames (ORFs)<br>The T-DNA of plasmid PHP27118 includes four expression cassettes for the genes cry1F, cry34Ab1, cry35Ab1 and pat. Short sequences of polylinker regions (which differ from the sequences of the plasmids PHP8999 and PHP17761 used for the generation of maize 1507 and maize 59122) are located between the individual expression cassettes. Bioinformatic analyses of the insert to look for putative ORFs and subsequent similarity searches of any ORF found within the insert to known allergens and toxins are missing. Therefore, complete bioinformatics analyses of the whole insert sequence using up-to-date databases should be delivered in addition. | Based on the information provided by the applicant, the insertion site is located in the upstream region of the hypothetical gene and thus, unlikely to have interrupted the coding sequence of the hypothetical GRX-like protein. Even if the insertion would have altered the expression of this predicted gene, there are no indications from comparative agronomic-phenotypic performance and compositional analyses of any unintended effect caused by the insertion.<br>Upon request of the GMO Panel, the applicant provided data on the bioinformatics analysis of all ORFs (additional information: 23/09/2015 and 28/09/2017). |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A, 3.2.1 Experimental design | Specifications on the purity of the starting material used in the field trials are missing in Annex 8_PHI-2011-001 and Annex 9_PHI-2012-031. Therefore, the applicant should provide data confirming the identity of maize 4114 (test material) and its absence in the control and reference material. | The GMO Panel takes note of the comment raised by Germany. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A, 3.4 Agronomic and phenotypic characteristics | The comparative analysis of agronomic and phenotypic characteristics does not include data on yield or yield components. As the EFSA Guidance Document (EFSA, 2011) as well as the Implementing Regulation (EU) No 503/2013 consider yield as an important agronomic and phenotypic endpoint, the applicant should explain why, in his opinion, those data are not necessarily needed to conclude on the risk assessment of maize 4114.<br><br>EFSA (2011). Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. [37 pp. doi:10.2903/j.efsa.2011.2150. Available online: www.efsa.europa.eu/efsajournal.htm | The GMO Panel noted that the supplied dataset on the agronomic and phenotypic characterisation of maize 4114 did not report data on yield or yield components (such as seed weight). The GMO Panel considers yield an important agronomic and phenotypic endpoint, as it enables characterisation of the plant's biology and performance. In accordance with the relevant EFSA GMO Panel's guidelines on the risk assessment of GM plants (EFSA GMO Panel 2010, 2011) and the Implementing Regulation (EU) No 503/2013, applicants are encouraged to provide data on yield and any other relevant yield components in support of the agronomic and phenotypic characterisation of the GM plants under assessment. The GMO Panel therefore requested the applicant to supply the missing data on yield for maize 4114 and any other relevant yield components.<br><br>The applicant provided on 23/9/2015 an additional study (Study Number PHI-2014-035) on the agronomic and phenotypic characterisation of maize 4114 maize performed at 8 sites in the US in the 2014 growing season and that contained the requested yield analysis among the originally assessed endpoints, as requested by the GMO Panel. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A, 4.5.1 Design and performance of 90-day feeding study in rodents | The applicant performed two repeated-dose 90-day oral toxicity studies with maize 4114 in rats. In both studies only one dose level of 32% (w/w) was tested<br><br>The study designs of the conducted 90-day oral toxicity studies in rats are not fully in line with the recommendations of EFSA (EFSA, 2011 and EFSA, 2014). EFSA (EFSA, 2011) recommended two dose levels (high dose and low dose) when testing whole food/feed. In this context, the high dose level should correspond to the highest level of the whole food/feed that can be incorporated in the animal diets whilst avoiding nutritional imbalances. The low dose level could be half to a quarter of the high dose and should always be above the anticipated human intake. However, according to an explanatory statement of EFSA (EFSA, 2014), in the absence of a test hypothesis (scenario 2) a scientifically justified option is to use only one dose level of the GM test material at the maximum incorporation rate.<br><br>The first 90-day feeding study with maize 4114 (Annex 20a_PHI-2011-055) is compliant to scenario 2 as no relevant changes and/or specific hazards were identified in the preceding evaluations. Therefore, the use of only one dose level of the GM test material is possible in principle provided that this dose level reflects the maximum incorporation rate. On the basis of current knowledge EFSA (EFSA, 2014) proposed an incorporation rate of 50% for maize as reference value for the high dose in 90-day studies in rodents. However, the applicant tested only 32% (w/w). | The GMO Panel noted that the applicant only tested one dose level in the first 90-day feeding study with maize 4114 (Annex 20a_PHI-2011-055). However the dose tested was close to the highest possible without inducing nutritional imbalance according to the current knowledge, and in accordance to the limit test dose as described in OECD TG 408. This is considered not to compromise the study (scientific opinion 3.4.1.4). Moreover, EFSA is aware that further investigation on the doses is undertaken by EU project (e.g. G-Twyst)<br><br>The purpose of the second 90-day feeding study (Annex 23_PHI-2013-232) is focused on the assessment on histopathology of the kidney, at the experimental condition of the main feeding study. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | Scenario 1 (specific hypothesis to be tested) applies for the second, renal-focused 90-day feeding study (Annex 23_PHI-2013-232) because in the first 90-day study two male rats in the 4114 group were diagnosed with bilateral, multiple renal tubule tumors. Again, the applicant tested only one dose level of 32% (w/w).<br><br>Although the studies may not meet the high requirements laid down by EFSA, the results of the second 90-day study support the conclusion of the Pathology Expert Working Group that the proliferative renal tubule cell lesions observed in the first study were spontaneous and not related to administration of the test diet containing maize 4114.<br><br>EFSA Scientific Committee; 2011. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 2011;9(12):2438 [21 pp.] doi:10.2903/j.efsa.2011.2438. Available online: www.efsa.europa.eu/efsajournal<br><br>EFSA, 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp., doi:10.2903/j.efsa.2014.3871 | |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 3.1. Persistence and invasiveness including plant-to-plant gene flow | The import documents should indicate that maize 4114 has not been approved for cultivation by the EC. In addition to the intended GM labelling, a clear labelling of maize 4114 indicating the tolerance to glufosinate ammonium 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 4114 about avoidance and control of spillage. | Labelling of maize 4114 is outside the remit of the GMO Panel. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4. Post-Market Environmental Monitoring (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 plan falls outside the mandate of EFSA. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.2. Case Specific Monitoring (approach, 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. | The GMO Panel takes note of the comment raised by Germany. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.3. General Surveillance (approach, method and analysis) | 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 which information will be pro-actively queried, gathered, and how they will be evaluated.<br><br>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. | 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.<br><br>No biologically relevant compositional, agronomic and phenotypic changes were identified in maize 4114 when compared with its non-GM comparator. The GMO Panel therefore considered maize 4114 to be as safe as the non-GM comparator and that post-market monitoring of the food and feed derived from maize 4114 is not necessary. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.3.1. Farmers' survey (for cultivation) and operators' survey (for Import and Processing) | The strategy of General Surveillance is mainly based 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. | 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 | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.3.2. 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. | 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 | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.3.3. Review of ongoing research and development activities and literature review | In general, other sources of information, e.g. peer-reviewed publications or ongoing research should be taken into account. However, the applicant should describe in detail how he will consider this information within 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. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | E, 4.3.4. Reporting | A report on General Surveillance 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. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| Germany | Federal Agency for Nature Conservation (BfN) | A. Hazard identification and characterisation | The Federal Agency for Nature Conservation (BfN) considers that further information is required before the risk assessment of EFSA/GMO/NL/2014/123 can be finalized. In particular the environmental risk assessment (e.r.a.) and the monitoring plan should be amended.<br><br>Information (data and data analyses) provided on phenotypic evaluation, composition, and toxicology is insufficient and conclusions of equivalence of maize 4114 and conventional maize and on food and feed safety based on this information are premature.<br><br>Phenotypic stability of the introduced traits in maize 4114 was studied over five separate generations by analyzing leaf samples using PCR and by evaluating plants that were treated with glufosinate. However, the performance of maize 4114 was not studied in the presence of the target organisms. Also, the degree of glufosinate resistance is not given, but would be relevant with regard to the field design for the comparative assessment.<br><br>The applicant's risk identification is largely focused on direct effects of the transgenic proteins (toxicity, allergenicity). Unintended effects due to the introduction of the four transgenes into the maize genome and due to residues of the complementary herbicide or its metabolites were neither taken into consideration nor were they assessed. However, they cannot be excluded and data should be provided.<br><br>Glufosinate ammonium induces severe reproductive and developmental toxicity (EFSA 2005). In the EU glufosinate ammonium was classified in Category 2 and 3 of reproductive toxicity with the risk phrases R60 ("May impair fertility") and R63 ("Possible risk of harm to the unborn child") (Commission Directive 2009/2/EC). It is expected that glufosinate ammonium will be phased out in the EU at the end of September in 2017 due to its reproductive toxicity (see Annex I of Commission Implementation Regulation (EU) No 540/2011).<br><br>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.<br><br>EFSA (2005). EFSA Scientific Report. Conclusion regarding the peer review of the pesticide risk assess-ment of the active substance glufosinate. 27, 1-81. | Based on the information provided in the frame of application EFSA-GMO-NL-2014-123 which includes also additional information, the GMO Panel was able to conduct the risk assessment of maize 4114.<br><br>The data provided by the applicant on the phenotypic and insert stability is in line with the applicable EFSA GMO Panel guidelines.<br><br>Unintended effects due to the introduction of the four transgenes into the maize genome were evaluated bioinformatically (e.g. interruption of endogeneous genes) and were in line with the applicable EFSA GMO Panel guidelines. The assessment of unintended effects due to residues of the complementary herbicide or its metabolites is not in the remit of the EFSA GMO Panel.<br><br>The GMO Panel takes note of the comments made by Germany.<br><br>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 | Federal Agency for Nature Conservation (BfN) | A, 3.2 Field trials: experimental design and statistical analysis | Field trials for comparative assessment including agronomic and compositional analyses were conducted at six sites in the United States in 2011 (Annex 8_ PHI-2011-001) and at three sites in the USA and one in Canada in 2012 (Annex 9_ PHI-2012-031). A total of six non-GM reference lines per field study (12 lines in total for both field studies) were also included in the study to provide a proper estimate of natural variability due to environmental factors and genetic variation. The GMO was treated with and without the complementary herbicide glufosinate. The experimental design has got several weak points:<br><br>I. According to the applicant (main text p.75) "The different sites selected for the field trials | The field trial design was in line with the recommendations outlined in the EFSA guidance (EFSA GMO Panel, 2011).<br><br>In addition to the field trials conducted in US and Canada in 2011 and 2012, respectively (Annex 8 and Annex 9), to assess the agronomic characteristics and nutrient composition of maize, the applicant provided an additional agronomic study performed in US in 2014 at eight sites (Study Number PHI-2014-035). |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | reflect the different meteorological and agronomic parameters under which the product is expected to be grown". However, justification is missing, whether locations are "representative of the range of receiving environments where the crop will be grown, thereby reflecting relevant meteorological, soil and agronomic conditions" (EFSA 2011, p.14). Comparing information and data on agronomic practices and on biotic stress (prevailing pest and disease pressure) are missing. States in the North or more towards the East of the USA (such as Minnesota, Michigan, Indiana, Ohio, Pennsylvania) were not considered as trial sites in the present application, but for application of other GM maize (EFSA-110, EFSA-113, EFSA-115 and EFSA-118). Therefore it is actually questionable, that the locations chosen in the present application are representative of the range of receiving environments.<br><br>II. While application of maintenance chemicals varied according to the various sites, glufosinate was applied solely at a uniform rate, not considering regional agronomic conditions. To our understanding rates of the complementary herbicide should also be case-specific and take into account the amount of active ingredient tolerated by a certain GMO. In this respect, data are missing and requested on the amount of glufosinate tolerated by maize 4114. (cf. comment under A.4. toxicology).<br><br>III. Ideally compositional and agronomic studies should be based on a full power analysis, conducted prior to finalising the design.<br><br>IV. The purity of the starting material used in Annex 8 and 9 was not sufficiently tested. A certificate of analysis is missing, confirming the absence of contamination with other GM maize lines in the used starting material.<br><br>V. The trial site description contains some relevant information, but history of pest management and present pest and disease infestation is missing.<br><br>VI. Interactions between environmental factors (climate, soil or agricultural practices) and the GMO were not analyzed.<br><br>The experimental design of field trials should be devoid of the above listed deficits. We recommend including data from field experiments from several years for the analysis to include climatic variation between years. These should – in accordance with the step-by-step principle – be supplemented by data from greenhouse studies, e.g. those already collected during breeding of maize 4114, which allows simulation of well-defined abiotic and biotic conditions.<br><br>EFSA (2011). Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. | At each site, the following materials were grown in a randomised complete block design with four replicates: maize 4114 not treated with the intended herbicide (maize 4114/not-treated), maize 4114 treated with glufosinate (maize 4114/treated), a non-GM comparator, and several commercial non-GM maize reference varieties (i.e. three in the 2011/2012 study and four in the 2014 study). All materials were treated (sprayed) with required maintenance pesticides (including conventional herbicides) according to local requirements. In total, 12 and 20 non-GM maize reference varieties were included across the field trial sites performed in 2011/2012 and 2014, respectively.<br><br>Field trials included data on monthly temperature, rainfall, and irrigation as well as on maintenance product applications. The field trials were conducted in major maize growing areas of the US and Canada, representing regions of diverse agronomic practices and environmental conditions. This was considered satisfactory by the GMO Panel.<br><br>The statistical analysis was in line with the requirements outlined in the EFSA guidance (EFSA GMO Panel, 2011).<br><br>The GMO Panel wants to add that guidance on the agronomic and phenotypic characterisation of GM plants was published on 24/6/2015 (EFSA GMO Panel, 2015). For all applications submitted 24 months or more after the publication date need to adhere to the requirements laid down in this guidance document are fully applicable. These requirements include a comprehensive and accurate description of various aspects of the receiving environments (such as geographical location, agrometeorological data, soil characteristics, cropping history, post-harvest conditions and crop management practices). |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| Germany | Federal Agency for Nature Conservation (BfN) | A, 3.3 Compositional analysis | For general comments on field trial design and comparative assessment we refer to A.3.2. The applicant should be asked to provide a robust and reliable data basis for the composition of maize 4114 to demonstrate substantial equivalence of 4114 and conventional maize, which is devoid of the deficits listed under A.3.2. In addition, the compositional analysis did comprise neither residues of the complementary herbicide nor its metabolites. This is of great relevance, because herbicide resistance conferred by genetic modification allows for a more intensive use of the complementary herbicides. Moreover, an analysis of the grain with regard to the herbicide applied and its metabolites is mandatory. | The GMO Panel takes note of the comment raised by Germany. Maize 4114 grains and forage harvested from the field trials in the North America in 2011/2012 were analysed for 84 constituents (9 in forage and 75 in grain). The analysis included the key constituents recommended by OECD (OECD, 2002).<br>The assessment of potential consumer health risks resulting from pesticide residues and metabolites in the GM maize is not in the remit of the GMO Panel. |
| Germany | Federal Agency for Nature Conservation (BfN) | A, 3.4 Agronomic and phenotypic characteristics | PART I<br>Results on agronomic characteristics and composition all refer to the same set of field trials. For general comments on comparative assessment and the production of material we refer to comments on A.3.2. Results about volunteers from field releases performed in various countries are not provided.<br>Further data and analysis are required before phenotypic and ecological equivalence can be concluded. Next to the weak points of the experimental design (cf. comments under A.3.2.) this is for the following reasons:<br>I. The selected agronomic characteristics cannot sufficiently indicate differences in dissemination and survivability of maize 4114 compared to conventional maize.<br>II. Data sets are based on a field design which is – because of the small plot size – not comparable to common agricultural practice. Pesticides were applied rarely or frequently depending on the site. It cannot be excluded that both aspects interfered with the collection of ecological interaction data (e.g. arthropod abundance).<br>III. As already requested by EFSA (Ref. EW/ZD/MA/shv, 11.3.2015) data on yield and yield components are entirely missing. These data are however important for the analysis of agronomic and phenotypic characteristics.<br>IV. According to the applicant (main text p.78) "Given the large diversity of growing conditions represented across the 10 sites and the characteristics of the newly expressed proteins, it is unlikely that other growing conditions (i.e. conditions not represented at the 10 sites) or additional sites would result in different conclusions than those presented based on data from 10 sites.". However, at least with respect to plant height and ear height this statement is questionable: Plant height and ear height has been uniformly been shown to be on average higher in the case CHT Maize 4114 and IHT Maize 4114 when compared with control Maize at the 10 test sites. Whereas in the case of the field production and characterization study that was performed in the context of the toxicology testing (Annex 21), these results are reverse. There the CHT Maize 4114 and IHT Maize 4114 is around 10% lower when compared with control Maize. The applicant is therefore asked to analyze these data as well and to put them | The applicant submitted field trials conducted in US and Canada in 2011 and 2012 to assess the agronomic and phenotypic characteristics of maize 4114 an additional field trial performed in US in 2014 on eight sites was submitted by the applicant, following a request from the GMO Panel to include the endpoint "yield".<br>Fourteen and fifteen agronomic and phenotypic endpoints were analysed in total in the 2011/2012 and 2014 field trials, respectively:<br>Early population, seedling vigour, time to silking, time to pollen shed, pollen viability and colour, plant height, ear height, stay green, disease incidence, insect damage, stalk lodging, root lodging, final population, and yield (only in the 2014 field trials).<br>The GMO Panel considered that the endpoints measured were sufficient to assess the agronomic and phenotypic characteristics of maize 4114.<br>For maize 4114/not-treated and/treated, the test of difference identified statistically significant differences for 'plant height' and 'ear height'. For these two endpoints, the test of equivalence indicated full equivalence to the set of non-GM reference varieties.<br>In addition to the field trials, seed characteristics of maize 4114 were also tested under controlled conditions. Seed germination of maize 4114 was compared with that of its |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | into relation to the agronomic studies. This is also underlining our request (see A.3.2.) to enlarge the number of sites and years in the agronomic testing, because some effects of the transgene can only emerge under specific local and environmental conditions.<br><br>V. Ecological interaction data are insufficient: data on abiotic stress such as cold, compaction, drought, flood, frost, hail damage, heat, nutrient deficiency, and wind damage are entirely missing and data on disease and pest were restricted to R 5 growth stage (cf. Annex 8 and 9). Comparing information and data on biotic stress (prevailing pest and disease pressure) are missing for the locations (cf. comment under A.3.2.).<br><br>VI. Only a single charge of GMO seed material was tested for germination. Therefore it was not tested – as suggested by EFSA (2011) – what influence different receiving environments or the complementary herbicides might have on the produced seed material that is assessed for germination. | non-GM comparator. Seeds were incubated under controlled conditions at three different temperature regimes and the numbers of germinated (normal and abnormal) and non-germinated (hard, imbibed and dead) seeds were counted. |
| Germany | Federal Agency for Nature Conservation (BfN) | A, 3.4 Agronomic and phenotypic characteristics | PART II<br><br>With regard to a final assessment, further information is required, because the information provided is not considered sufficient to support the conclusion of a substantial equivalence of maize 4114 to conventional maize, which is the basis of further conclusions in application EFSA/GMO/NL/2014/123.<br><br>The applicant should be asked to provide a robust and reliable data basis for reproduction, dissemination, and survivability to demonstrate substantial equivalence of maize 4114 and conventional maize. Field studies with ecology-based parameters such as frost tolerance, seed dormancy or time span of pollen emission and relevant interaction data towards abiotic and biotic stress of maize 4114 tested under field conditions should be included in the application. Data should account for several locations and growing season, e.g. a minimum of three growing seasons and six locations representing different environmental conditions. The statistical power of analyses should be given. Criteria on which the representativeness of locations has been established should be given and the environmental conditions should be documented and provided with the application to assess their possible effects on the considered parameters. We recommend including data on the occurrence of volunteers during cultivation of the GMO at all sites. In agreement with the 'step by step' principle field results including post-release monitoring reports from the releases of maize 4114 shall be provided.<br><br>EFSA (2011). Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150. | The applicant collected endpoints during early, mid and late season, providing a complete description of the life cycle of the crop during the different growth stages. Considering the scope of the application, that does not cover cultivation, the GMO Panel considered sufficient the information provided by the applicant to evaluate the agronomich and phenotypic characteristics of maize 4114. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| Germany | Federal Agency for Nature Conservation (BfN) | A, 4. Toxicological assessment | The whole plant feedings studies are a substantial and integral part of the toxicological assessment, because in this model systhemic effects caused by the transgene or the transformation can be evaluated. Therefore the presented data are an important data set.<br><br>However the thirteen-week feeding studies in rats (Annex 20a_PHI-2011-055 and Annex 23_PHI_2013-232) have got some weak points which compromise the conclusions: (i) The studies did not use two different dosages of test material as required by Implementing Regulation (EU) 503/2013; or alternatively the dosis of 50% according to the EFSA (EFSA, 2014) (ii) neither the test material (test and control maize corn) nor the basal LabDiet 5002, which contains corn as main ingredient, were analysed for contamination with GM material (apart from the test event and the reference events). Given the high percentage of GM corn and soy grown in the US a proof of the absence of further GM material is essential.<br><br>The applicant's claim that plants were grown under typical agricultural practices for commercial U.S. corn production has not been specified and should be demonstrated especially for glufosinate, which was applied to maize 4114. Rates should relate to normal application rates of glufosinate for HR crops at the various trial sites. To our understanding rates of the complementary herbicides should also be case-specific and take into account the amount of active ingredient tolerated by a certain GMO. In this respect, data are missing and requested on the amount of glufosinate tolerated by maize 4114 (cf. comment under A.3.2.). In addition analysis of glufosinate-residues in the test diet is required.<br><br>In addition to the 90-day feeding study in rodents, we advise to carry out supplemental studies with ruminants and swine which differ with respect to their digestive systems and which will be substantially exposed to feed derived from maize 4114.<br><br>EFSA, 2014. Explanatory statement for the applicability of the Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed for GMO risk assessment. EFSA Journal 2014;12(10):3871, 25 pp., doi:10.2903/j.efsa.2014.3871<br><br>Séralini, G.E., Clair, E., Mesnage, R., Gress, S., Defarge, N., Malatesta, M., Hennequin, D., de Vendomois, J.S., 2012. Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem. Toxicol. 50, 4221–4231. | The GMO Panel noted that the applicant only tested one dose level in the first 90-day feeding study with maize 4114 (Annex 20a_PHI-2011-055). However the dose tested was close to the highest possible without inducing nutritional imbalance according to the current knowledge, and in accordance to the limit test dose as described in OECD TG 408. This is considered not to compromise the study (scientific opinion 3.4.1.4). Moreover, EFSA is aware that further investigation on the doses is undertaken by eu project (e.g. G-Twyst). Check for contamination with GM material apart from the test event and the reference events is not a mandatory requirement.<br><br>A description of field production of grains used in the study was submitted in the context of the technical dossier; please refer to Annex 21 PHI-2010-080. The assessment of potential consumer health risks resulting from pesticide residues and metabolites in the GM maize is not in the remit of the GMO Panel.<br><br>No substantial modifications in the composition of maize 4114, no indication of possible unintended effects and no indication of interactions relevant for food/feed safety were identified. Therefore, animal studies on food/feed derived from maize 4114 are not considered necessary by the EFSA GMO Panel. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| Germany | Federal Agency for Nature Conservation (BfN) | B. Exposure assessment - Anticipated intake/extent of use | Water and soil organisms may be exposed to maize 4114 via the release of organic waste material, litter or sewage to the environment, which occurs during processing, use or through spillage. No data are provided by the applicant about the concentration of the proteins Cry1F, Cry34Ab1, Cry 35Ab1 in organic waste material, litter or sewage and how this is a pathway of exposure of the environment. The possibility of an accumulation of the mentioned substances in the environment and of subsequent effects on water and soil organisms should be assessed and consider that waste and litter from several GM maize and GM soybean lines (MON87701; MON87701xMON89788; DAS-81419-2) containing Cry proteins might enter the environment. Therefore, the applicant is requested to provide data on this issue and to submit a risk assessment concerning the possible exposure of water and soil organisms to the mentioned substances. | Considering the scope of application EFSA-GMO-NL-2014-123, environmental exposure of water and soil organisms to spilled maize 4114 grains or occasional feral GM maize plants arising from spilled GM grains is limited. Therefore, potential interactions of the GM plant with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. |
| Germany | Federal Agency for Nature Conservation (BfN) | D. Post Market Monitoring (PMM) of food and feed derived from GM plants | The data provided to show the human and animal safety of maize 4114 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.<br>The applicant is further requested to explain how the PMM of maize 4114 in mixed GMO commodities imported, processed or used for food/feed is realized. 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 the EU approved GM maize and consequently all parameters identified for the different GM maize products should then be monitored. | The food/feed products derived from maize 4114 are as safe and nutritious as those derived from the non-GM comparator. Therefore, the GMO Panel considers that the post-market monitoring of the food and feed derived from maize 4114 is not necessary (see section 3.4.7 in the opinion). |
| Germany | Federal Agency for Nature Conservation (BfN) | E. ERA | The Federal Agency for Nature Conservation (BfN) considers that further information is required before the risk assessment of EFSA/GMO/NL/2014/123 can be finalized. The environmental risk assessment (e.r.a.) should be amended subjected to the required further information. | The GMO Panel is of the opinion that, considering the scope of application EFSA-GMO-NL-2014-123, the information provided was in line with the requirements outlined in the EFSA guidance on environmental risk assessment of GM plants (EFSA GMO Panel, 2010) and was sufficient to carry out the environmental risk assessment of maize 4114. |
| Germany | Federal Agency for Nature Conservation (BfN) | E, 4. Post-Market Environmental Monitoring (PMEM) | 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).<br>The monitoring plan has to be elaborated in more detail in order to meet the following requirements:<br>• Provision of a fully specified list of monitoring parameters.<br>• Application of standardized sampling methodologies: A basic prerequisite for comparing GMO | Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.<br>As the ERA did not identify potential adverse environmental effects from the maize 4114, no case-specific monitoring is required. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | 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 http://www.vdi.eu/engineering/vdi-standards/.<br>• Elaboration of a sampling concept.<br>• 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.<br>• Elaboration of the methods of data analysis including the statistical methods.<br>• 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).<br>The monitoring should be run in regions, where viable maize 4114 will be transported, stored, packaged, processed or used for food/feed. In case of substantial losses and spread of maize 4114 all receiving environments need to be monitored.<br>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.<br>Since traders may commingle maize 4114 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 maize 4114 and those caused by other GM maize.<br>The Federal Agency for Nature Conservation is of the opinion that a detailed monitoring plan has to be provided before consent may be given.<br>Bartz, R., Heink, U. and Kowarik, I. (2009). Proposed Definition of Environmental Damage Illustrated by the Cases of Genetically Modified Crops and Invasive Species. Conservation Biology 24 (3): 675–681. DOI: 10.1111/j.1523-1739.2009.01385.x<br>EFSA (2011). Scientific opinion. Guidance on the Post-Market Environmental monitoring (PMEM) of genetically modified plants. EFSA Journal, 9(8): 2316, 40 pp. | |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| Germany | Federal Agency for Nature Conservation (BfN) | E, 4.1. Interplay between Environmental Risk Assessment and PMEM | The information necessary to conclude on the ERA is partly missing. Thus, the safety of maize 4114 cannot be fully assessed. Depending on those results the conclusions concerning case-specific monitoring may need to be revised. | The GMO Panel takes note of the comment raised by Germany. |
| Germany | Federal Agency for Nature Conservation (BfN) | E, 4.2. Case Specific Monitoring (approach, method and analysis) | 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 maize 4114 or material containing maize 4114 enters the environment. The applicant is requested to provide an appropriate case-specific monitoring plan comprising at least the following elements:<br>i.) spillage or loss of maize 4114 during transport, storage, packaging, processing and use (feed and food),<br>ii.) potential spread and persistence of maize 4114, if spillage or loss of viable maize 4114 occurs,<br>iii.) exposure of the Cry1F, Cry34Ab1 and Cry35Ab1 proteins to the environment e.g. via sewage water, waste material or by-products which occur during processing or use of non-viable maize 4114 material as food/feed;<br>iv.) environmental effects such as spread, persistence and accumulation of Cry1F, Cry34Ab1 and Cry35Ab1 proteins in other organisms and environmental media;<br>v.) if spread and persistence of the Cry1F, Cry34Ab1 and Cry35Ab1 proteins occur, further observations of impacts on organisms, food chains, and habitats are required;<br>Maize 4114 may enter the environment together with other approved GM maize lines containing different Bt proteins. Therefore, a special focus should be on combined effects.<br>For these parameters, the use of the following methods is recommended (http://www.vdi.eu/engineering/vdi-standards/):<br>o VDI-Guideline 4330 Part 10 "Floristic mapping of genetically modified plants their crossing partners and their hybrid offspring"<br>o VDI-Guideline 4330 Part 5 "Guideline for the collection and preparation of plant samples for molecular biological analysis"<br>If risk management measures are envisaged, e.g. to minimize incidental spillage during transport, storage, packaging or processing, their efficacy should be monitored during case-specific monitor-ing (EFSA 2011).<br>VDI (2011). VDI Guidelines: monitoring the ecological effects of genetically modified organisms. Genetically modified plants. http://www.vdi.eu/engineering/vdi-standards/<br>EFSA (2011). Scientific opinion. Guidance on the Post-Market Environmental monitoring | As the environmental risk assessment did not identify potential adverse environmental effects from the maize 4114, no case-specific monitoring is required. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | (PMEM) of genetically modified plants. EFSA Journal, 9(8): 2316, 40 pp. | |
| Germany | Federal Agency for Nature Conservation (BfN) | E, 4.3. General Surveillance (approach, method and analysis) | 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 operators handling and using viable maize 4114 and reported to the authorization holder, represented by EuropaBio. It remains unclear, how the authorisation holder/EuropaBio will inform operators about their surveillance function and how it will be assured that operators in duty for general surveillance show the necessary skills to detect environmental impacts of maize 4114.Therefore, the applicant is requested<br>• to name the national and local organisations and factories involved in the monitoring,<br>• 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,<br>• 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.<br>In case the suggested operators are not capable to cover all relevant observation objects, further monitoring systems have to be established.<br>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.<br>The general surveillance plan has to focus on possible pathways how maize 4114 can get into the broader environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal and use of maize 4114. 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 viable maize 4114, during transport, storage, packaging, processing and use.<br>Maize 4114 may enter the environment together with other approved GM maize lines. Therefore, a special focus should be on possible combined effects. | Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitorin plan falls outside the mandate of EFSA. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| Germany | Federal Agency for Nature Conservation (BfN) | E, 4.3.4. Reporting | 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.<br>The monitoring report should also deliver detailed information on<br>i) actual volumes maize 4114 imported into the EU,<br>ii) the ports and silos where shipments of maize 4114 were unloaded,<br>iii) the processing plants where maize 4114 was transferred to,<br>iv) the amount of maize 4114 used on farms for feed, and<br>v) transport routes of maize 4114.<br>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 PMEM plan falls outside the mandate of EFSA. |
| Hungary | Ministry of Agriculture | A, 1. Information relating to the recipient or (where appropriate) parental plants | Further information should be added on what exactly is the "Region required for cloning genetic elements"?<br>It is stated that "The *cry1F*, *cry34Ab1*, *cry35Ab1*, and *pat* genes and regulatory elements inserted in 4114 maize are the same as the sequences inserted in 1507 and 59122 maize since 2003, 2006, and 2006, respectively. 1507, 59122, and 1507x59122 maize contain familiar traits and are currently licensed broadly. Therefore, all constituents' fragments of the region intended for insertion have a history of safe use in food and feed". We do not agree with using any GM plant for 2-12 years counts as "history of safe use".<br>"The codon-optimised *cry1F* gene introduced is identical to the *cry1F* gene introduced in 1507 maize which has already been approved." The authorisation of maize 1507 was objected on a scientific basis.<br>Bacillus thuringiensis (Bt) has not been directly used for food and feed, so there is no historic safety with those bacteria/products either.<br>The "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 (US-EPA, 1998a)", but those microbial preparations have never been consumed as food or feed either. | A 'polylinker' is a widely used term in molecular biology. Polylikers are normally short DNA sequences introduced into vectros to fascilitate cloning.<br>The GMO Panel takes note of the comments made by Hungary.<br>The GMO Panel takes note of the comments made by Hungary. |
| Hungary | Ministry of Agriculture | A, 2.1 Information relating to the genetic modification | The statement "CaMV is naturally present on many vegetables and it is likely that humans have had long exposure to the virus. No adverse effects have been reported through ingestion of CaMV infected food or feed" is incorrect. Humans or animals are exposed to the intact virus via food/feed. The genetic elements of the intact virus are covered by the coat protein(s) to which human cells have no receptors for. The genetic elements (DNA) in the GM plant are "naked", not covered by a protein coat, and therefore have no "specificity". They are able to drive transgene expression in several species, including mammalian cells. In addition, the | The GMO Panel considers that the potential gene expression induced by the 35S CaMV promoter present in maize 4114 does not present a risk per se, in animal cells. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| | | | genetic elements of the CaMV are in a different "molecular environment" than in the native virus.<br><br>It is stated on page 30 that "None of the gene encoded proteins in 4114 maize has been shown to have any relationship with toxins, anti-nutrients or allergens (EFSA, 2009a). This is confirmed on the basis of new bioinformatics similarity searches against up-to-date sequence databases, as discussed in Sections 1.4.1 and 1.5.1."<br><br>Further information should be added on what the term Cry toxin is referred to? ("The Cry1F protein belongs to the 3-domain family of δ-endotoxins produced by the bacterium Bt (Bravo et al., 2007; de Maagd et al., 2003; Pigott and Ellar, 2007)). The Cry toxins do belong to the A - B type toxin family, just as does the microbial cholera toxin or the plant toxin ricin.<br><br>Further information should be added on the evidence that mammalian cells do not have receptors for Cry toxins. According to Mizuki et al., (1999) mammalian cells are killed by Cry toxins (Mizuki, E, Et Al., (1999) Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell- killing action on human cancer cells. J. Appl. Microbiol. 86: 477–486.).<br><br>All Cry protein genes inserted to the GM maize plants are synthetic-, and modified (codon optimized) versions of the genes occurring naturally in Bt bacteria. Therefore, instead of the natural or recombinant bacterial versions of the same gene(s)/protein(s) the actual transgene(s) and the transgenic protein(s) isolated from the GM plant, need to be used in the experiments and/or examined and proved to be safe.<br><br>Up until now no experimental evidence was provided to prove that human/mammalian cells do not have receptors to which Cry proteins can attach themselves to. | Cry1F, Cry34Ab1 and Cry35Ab1 proteins were previously assessed by the GMO Panel and no safety concerns for humans and animals were identified. Updated bioinformatics analysis did not reveal similarities to known toxins. The GMO Panel is not aware of any new information that would change previous conclusions (see section 3.4.3.1 of the opinion).<br><br>The GMO Panel takes note of the comments made by Hungary.<br><br>Please see reply above. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

| Country | Organization | Reference | Comment | GMO Panel response |
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| Hungary | Ministry of Agriculture | A, 2.2 Information relating to the GM plant | It is stated that "Western blot analysis was used to demonstrate that the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins in 4114 maize migrate with equivalent molecular weight and similar relative immunoreactivity to the proteins expressed in 1507x59122 maize". However, migration of DNA/proteins in SDS page is just not sensitive enough to show equivalence of molecular weights.<br><br>Although the expression of the insert encoded proteins was compared between 4114 maize,1507, 59122 and 1507x59122 maize in the same field trials, but the concentrations of the Cry toxin and PAT proteins were different in the different varieties. Therefore previously submitted food/feed safety data for Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins are not applicable for 4114 maize, since the comparisons revealed that 4114 maize tissues have dissimilar concentrations of the introduced proteins - except for the Cry1F and Cry34Ab1 protein concentrations in senescent tissue, and for Cry1F protein concentrations in pollen – than those of 1507, 59122, and/or 1507x59122 maize varieties. | The GMO Panel acknowledges the limitations of an SDS-PAGE/western blot analys. Howerver the provided data were considered adequate to indicate the similar migration and immunoreactivity of the Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins.<br><br>Although comparisons in the expression levels of Cry1F, Cry34Ab1, Cry35Ab1, and PAT proteins are presented in the application, 4114 maize is a single event and expression levels of the newly expressed proteins were therefore assessed independently of the previously assessed 59122, 1507 and1507x59122 GM plants. |
| Hungary | Ministry of Agriculture | A, 2.2.2 Information on the sequences actually inserted/deleted or altered | Fig. 1.2.11, Fig. 1.2.12, Fig. 1.2.13, Fig. 1.2.14, Fig. 1.2.19 c:<br><br>Further information should be added on why is it that 3 and 1 copy of PHP27118 + PHWWE shows lanes in a different position than 4114 maize /T2 (F1*1 generation) and 4114 maize /T3 (F1*1 generation)? | Taking into account the probes and restriction enzymes used as well as the limitations of the Southen methodology, the GMO Panel considers that the provided data are adequate to conclude on the molecular characterisation of maize 4114. |
| ITALY | Ministero dell'Ambiente | E, 4.3. General Surveillance (approach, method and analysis) | General surveillance for unanticipated adverse effects -Approach. 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 DP-ØØ4114-3 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 PMEM plan falls outside the mandate of EFSA. |

**Application EFSA-GMO-NL-2014-123 (maize 4114)**

**Comments and opinions submitted by Member States during the three-months consultation period (Annex G)**

**Comments from National Competent Authorities under Directive 2001/18/EC**

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| ITALY | Ministero dell'Ambiente | E, 2. General approach of the ERA | ERA for DP-ØØ4114-3 maize<br><br>The ERA has been conducted according to the recommendations outlined in the last EFSA Guidance on the ERA of GM plants (EFSA Journal (2010) 8, pp. 1-111). Nevertheless, we retain that there are some incongruities or misunderstandings in the application of the six-steps approach of the ERA. In particular, we note some confusion among the steps for the specific areas of risk "Persistence and invasiveness including plant-to-plant gene flow", "Plant to micro-organisms gene transfer" and "Interactions of the gm plant with non-target organisms (NTOS)": indeed, in the step 1 (Problem formulation), the notifier should identify the hazard and not characterize the risk, and so the conclusions such as "the risk is therefore negligible", in this step, are inappropriate. It is required to review the ERA and the application of the six-steps approach.<br><br>Reference:<br><br>EFSA Panel on Genetically Modified Organisms, 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. | The GMO Panel takes note of the comment made by Italy. |
| ITALY | Ministero dell'Ambiente | E, 4. Post-Market Environmental Monitoring (PMEM) | Monitoring plan for DP-ØØ4114-3 maize conforming with Annex VII to Directive 2001/18/EC and Decision 2002/811/EC<br><br>• For this PMEM, the applicant took into account, in adding to the aforementioned legislation, also to the EFSA guidance on presentation of applications provided in the 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 (2006) 99, pp. 1-100). It is required to take into consideration also the EFSA guidance on PMEM, published in 2011 (EFSA Journal 2011;9(8):2316).<br><br>• 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 DP-ØØ4114-3 maize: it is required to provide such guidelines to evaluate their effectiveness.<br><br>• The applicant states that the routine surveillance is based on the HACCP principles as outlined in Annex I, but there isn't any annex to the PMEM: it is required to add this Annex.<br><br>• The authorization holder 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. Among these there are COCERAL, UNISTOCK and FEDIOL. The links related to COCERAL and UNISTOCK (www.coceral.com/.../227870, www.coceral.com/.../232602) are not working: it would therefore necessary to update these links. In addition, as a result of control on the official websites of the three associations (www.coceral.com/, www.unistock.be/, www.fediol.be/.../f1.html), in the Members section, we see that not all European countries are represented within these associations: therefore, it is required to provide a list of Member States not represented and explanations on the monitoring methodology to be adopted in | Monitoring is related to risk management, and thus a final adoption of the post-market environmental (PMEM) plan falls outside the mandate of EFSA. However, the GMO Panel gave its opinion on the scientific rationale of the PMEM plan provided by the applicant. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize 4114. The GMO Panel agreed with the reporting intervals proposed by the applicant in its PMEM plan. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| | | | them. (.....) | |
| ITALY | Ministero dell'Ambiente | E, 4. Post-Market Environmental Monitoring (PMEM) | (....) In addition to the aforementioned existing monitoring systems conducted by third parties, the notifier will perform a screening of peer-reviewed scientific publications relevant to the specific GMO: it is required to provide a report of this literature search, or to enter it in the annual monitoring report.<br><br>• Update the EuropaBio link (www.europabio.org/.../, that is not correct.<br><br>• 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 guidance of EFSA on PMEM (EFSA Journal 2011;9(8):2316) 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.<br><br>• 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 DP-ØØ4114-3 maize can be established": we ask to specify the investigation method.<br><br>• Finally, as described by the EFSA guidance, "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 are 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.<br><br>References:<br><br>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. | Monitoring is related to risk management, and thus a final adoption of the post-market environmental (PMEM) plan falls outside the mandate of EFSA. |
| The Netherlands | Ministry of Infrastructure and the Environment | A. Hazard identification and characterisation | The NL has assessed the dossier with respect to the environment safety of event 4114 maize and has no comments or requests for additional information in relation of the safety of the GM event. | The EFSA GMO Panel thanks The Netherlands for this comment. |

| Application EFSA-GMO-NL-2014-123 (maize 4114)<br>Comments and opinions submitted by Member States during the three-months consultation period (Annex G) | | | | |
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| **Country** | **Organization** | **Reference** | **Comment** | **GMO Panel response** |
| The Netherlands | Ministry of Economic Affairs and Ministry of Health, Welfare and Sport | A, 4. Toxicological assessment | The Dutch assessors are of the opinion that no 90-day toxicity and animal nutrition studies were required as analyses performed showed that there are no relevant changes in composition or otherwise that could compromise the safety or nutritious characteristics of this genetically modified maize. In order to comply with the requirement in Regulation (EU) No 503/2013 two 90-day oral toxicity studies in rats were, however, performed for this application. Furthermore a nutritional study in broilers was provided that was also deemed to have no added value for the nutritional assessment of the new GM plant variety. | The EFSA GMO Panel thanks The Netherlands for this comment. |

## References


EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. 111 pp. doi:10.2903/j.efsa.2010.1879

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