Country	Organization	Reference	Topic	Comment	GMO Panel responses
Austria	Fed.MinistryLabour/Soc.A/Health	2.3.2. Updated bioinformatics	AUT Comment_01	Appendix 2.3.2-08 Basic Local Alignment Search Tool for Nucleotides (BLASTN) Analyses of the Insert Sequence Using Microbial Databases The applicant is of the opinion that "Cry1Ab is unlikely to be expressed optimally in microbes due to the use of the viral 35S promoter." We would like to indicate that for bacterial transformation a concomitant transfer of bacterial promoters is no prerequisite for gene expression (Lorenz and Wackernagel 1994). The fate of the pat insert after DNA transfer to soil or gut bacteria and its effect on bacterial communities under glufosinate selection pressure is not discussed by the applicant. Please provide the missing information. Glufosinate is interfering with bacterial growth and is acting as antimicrobial agent under certain circumstances leading to shifts in bacterial community structures (Calanduoni and Villafranca 1986; Bartsch and Tebbe 1989; Ahmad and Malloch 1995; Sessitsch et al. 2005; Chau-Ling et al. 2007; Pampulha et al. 2007; Tothova et al. 2010; Kopcáková et al. 2015). Glufosinate may, therefore, act as potent selector for the acquisition of plant-derived transgenic pat homologs. We would like to ask the EFSA GMO Panel to take this into consideration. BLASTN analysis of the insert sequence against microbial sequences stored in the NCBI database was performed using the megablast algorithm.	The applicant submitted updated bioinformatic HGT analysis using up-to-date databases (spontaneous information 16/04/2020). The analysis was performed according to EFSA guideline (EFSA, 2017). Given the results of this analysis and that the recombinant DNA in maize Bt11 does not confer selective advantages to microorganisms, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT. The GMO Panel takes note of the comment. It should be noted that the assessment of the effect of the herbicides in the environment is not in the remit to the GMO Panel.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Homology search was performed according the parameters recommended by EFSA. The NCBI Microbes database used was from December 2017 and, thus, contained at time an up-to-date sequence collection. The applicant reports that the tested bacterial database contained 10551 complete bacterial sequences. It is doubtful that this small number can provide meaningful results concerning the potential for horizontal gene transfers and homologous recombination between incoming transgenic DNA and indigenous bacteria in natural environments. Considering the fact that it was estimated that for instance 1 g of soil may contain 10,000 (Torsvik et al. 2002) to more than 10 million different bacterial species (Gans et al. 2005) this bioinformatic approach covered only a negligible fraction of bacterial genomes which may serve as potential recombination partners in natural environments. Moreover, genomes even of the same bacterial species show substantial sequence variability, are highly dynamic and appear to be in a constant genetic flux (compare for instance the propensity of microbes to form different strains or to acquire or loose pathogenicity islands), which is clearly not sufficiently reflected in the present microbial databases of GenBank (Schmidt and Hensel 2004; Myers et al. 2006). At the present stage the relevance of the draw conclusions for estimating a potential risk derived from horizontal gene	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				transfer events by homology searches against currently available microbial sequence data collections are probably highly questionable but certainly affected by a high degree of uncertainty. This shortcoming is to be documented during the risk assessment process to facilitate an informed decision-making process for the involved risk managers (EFSA Scientific Committee 2016). The identified homologous sequence (i.e. "region 1") contains more than 1088 bp and, thus, provides an impressive anchor sequence for homology directed illegitimate recombination (HDIR). HDIR requires a single homologous anchor sequence of approx. 150 - 180 bp and a short region of microhomology of ca. 3-10 bp of incomplete sequence identity at the opposite end of the incoming DNA strand to mediate the insertion of foreign completely non-homologous to the recipient genome (de Vries and Wackernagel 2002; Prudhomme et al. 2002). These are substantially more relaxed conditions as presented by the applicant (2 x 95% sequence identity of 200 bp). By applying the stringent requirements for successful horizontal gene transfers as performed by the applicant only high frequency transfers of genes or gene fragments between plant and bacterial DNA would be accessible. The applied bioinformatic strategy is thus prone to deliver false negative results due to obvious insensitivity. HDIR is indeed assumed to be a rare event.	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				However, under selection pressure this rare event is rapidly fixed in the exposed bacterial populations (Townsend et al. 2012; Nielsen et al. 2014). We would like to indicate that extremely rare transformation frequencies are not predictive to exclude long term adverse events (Pettersen et al. 2005). We would like to ask the EFSA GMO Panel to take this into consideration. [Ahmad I, Malloch D, 1995. Interaction of soil microflora with the bioherbicide phosphinothricin. Agriculture, Ecosystems and Environment 54(3): 165-174. Bartsch K, Tebbe CC, 1989. Initial steps in the degradation of phosphinothricin (glufosinate) by soil bacteria. Appl Environ Microbiol 55(3): 711-716. Calanduoni JA, Villafranca JJ, 1986. Inhibition of Escherichia coli glutamine synthetase by phosphinothricin. Bioorg. Chem. 14: 163-169. Chau-Ling H, Chiu-Chung Y, Ching-Yuh W, 2007. Screening and Identification of Glufosinate-Degrading Bacteria from Glufosinate-Treated Soils. Weed Sci 55(6): 631-637. de Vries J, Wackernagel W, 2002. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				illegitimate recombination. Proc Natl Acad Sci U S A 99(4): 2094-2099. EFSA Scientific Committee, 2016. Guidance on uncertainty in EFSA scientific assessment. Draft. EFSA Journal. https://www.efsa.europa.eu/sites/default/files/consultation/150618.pdf (last access: 7.8.2017). Gans J, Wolinsky M, Dunbar J, 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309(5739): 1387-1390. Kopcáková A, Legáth J, Pristaš P, Javorský P, 2015. Already a short-term soils exposure to the field-rate glufosinate concentration significantly influences soil bacterial communities. Soil and Water Research 10(4): 271-277. Lorenz MG, Wackernagel W, 1994. Bacterial gene transfer by natural transformation in the environment. Microbiol Mol Biol Rev 58: 5563-5602. Myers GS, Rasko DA, Cheung JK, Ravel J, Seshadri R, DeBoy RT, Ren Q, Varga J, Awad MM, Brinkac LM, Daugherty SC, Haft DH, Dodson RJ, Madupu R, Nelson WC, Rosovitz MJ, Sullivan SA, Khouri H, Dimitrov GI, Watkins KL, Mulligan S, Benton J,	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Radune D, Fisher DJ, Atkins HS, Hiscox T, Jost BH, Billington SJ, Songer JG, McClane BA, Titball RW, Rood JI, Melville SB, Paulsen IT, 2006. Skewed genomic variability in strains of the toxigenic bacterial pathogen, Clostridium perfringens. Genome Res 16(8): 1031-1040. Nielsen KM, Bohn T, Townsend JP, 2014. Detecting rare gene transfer events in bacterial populations. Front Microbiol 4: 415. Pampulha ME, Ferreira MASS, Oliveira A, 2007. Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. J Basic Microbiol 47(4): 325-331. Pettersen AK, Bohn T, Primicerio R, Shorten PR, Soboleva TK, Nielsen KM, 2005. Modeling suggests frequency estimates are not informative for predicting the long-term effect of horizontal gene transfer in bacteria. Environ Biosafety Res 4(4): 223-233. Prudhomme M, Libante V, Claverys JP, 2002. Homologous recombination at the border: insertion-deletions and the trapping of foreign DNA in Streptococcus pneumoniae. Proc Natl Acad Sci U S A 99(4): 2100-2105. Schmidt H, Hensel M, 2004. Pathogenicity Islands in Bacterial Pathogenesis. Clin Microbiol Rev 17(1): 14-56.	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Sessitsch A, Gyamfi S, Tscherko D, Gerzabek M, Kandeler E, 2005. Activity of microorganisms in the rhizosphere of herbicide treated and untreated transgenic glufosinate-tolerant and wildtype oilseed rape grown in containment. Plant Soil 266: 105-116. Torsvik V, Ovreas L, Thingstad TF, 2002. Prokaryotic Diversity--Magnitude, Dynamics, and Controlling Factors. Science 296(5570): 1064-1066. Tothova T, Sobekova A, Holovska K, Legath J, Pristas P, Javorsky P, 2010. Natural glufosinate resistance of soil microorganisms and GMO safety. Central European Journal of Biology 5(5): 656-663. Townsend JP, Bohn T, Nielsen KM, 2012. Assessing the probability of detection of horizontal gene transfer events in bacterial populations. Front Microbiol 3: 27.]	
Austria	Fed.Ministry_ Labour/Soc.A /Health	2.3.3. Additional documents or studies performed by or on behalf of the applicant	AUT Comment_ 02	List of Syngenta studies performed within the period of 10 years - 90-day toxicity study It is acknowledged that the notifier provided the results of a 90-day oral toxicity study of GM maize Bt11 in Han Wistar rats (Study Report “6_90d rat_38506_Bt11.pdf”). The toxicity study was carried out to test if consumption of Bt11 resulted in any adverse effects as compared to the non-transgenic control maize. The notifier includes a	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				discussion on the observed statistically significant differences. However, it is unclear, why in the summary tables for bodyweight (g), haematology, clinical chemistry, etc. the number of animals per group is five (N = 5). (Please compare: Study Report, p. 449 ff. - Appendix 25). Is that a mistake? It is understandable that food consumption is measured per cage, but other parameters are measured per animal (with N = 10). It would be helpful if this point could be clarified. We would also like to point out that EFSA Guidance explains that if histopathological differences between test and control groups are observed, those from other groups should be examined as well (EFSA (2011), p. 11). The notifier mentions in the Study Report (p. 43) that "in one male (4002) there were minimal mixed inflammatory cell infiltrates within the mucosa of the stomach and in another male (4009) there was minimal submucosal granulomatous inflammation in larynx." The notifier did not examine animals of the low dose groups 1 and 3 with respect to those parameters. [EFSA, 2011. Guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. The EFSA Journal 9(12):2438: 1-21.]	The sample size (N=5) reported in the summary tables is the number of cages: the summary statistics were calculated using the cage as experimental unit, i.e. based on the mean values per cage. The GMO Panel considered that this choice was acceptable, as it is consistent with the choice done for the statistical analysis, and also considering that individual animal data were available (in the report and in the accompanying raw data files) for any additional investigation. The microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet including maize Bt11 at 41.5%. Moreover, there was a difference in the preparation of the 10% and 41.5% diets, which prevented a statistical comparison using both groups (Scientific Opinion, Appendix C). Therefore, no indication to assess histopathology in animals of the low dose groups with respect to those parameters.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
Austria	Fed.Ministry_ Labour/Soc.A /Health	2.3.3. Additional documents or studies performed by or on behalf of the applicant	AUT Comment_ 03	Sequence information We appreciate the applicant's approach to re-sequence the transgenic Bt11 insert and flanking region sequences for the present renewal application. However, the sequencing data used for the alignment studies in microbial databases is nevertheless more than 6 years of age (please see Appendix 2.3.2 08, page 9 - Forrester, 2012).	The GMO Panel takes note of the comment. It should be noted that the sequencing study was submitted as additional study. The applicant clarified that the sequence of the event Bt11 is identical to the sequence of the event originally assessed.
Austria	Fed.Ministry_ Labour/Soc.A /Health	4. Monitoring plan and proposal for improving the conditions of the original authorisation	AUT Comment_ 04	Part II: Specific information, p. 27 We appreciate the applicant's efforts to describe the quality criteria and problems associated with sample drawing and DNA extraction and purification of these samples. In this context the applicant is using the abbreviation "DDGS". This abbreviation is missing in the list of abbreviations on page 7. Could you please explain the meaning of this letter code?	The term DDGS refers to 'distillers' dried grains and solubles'.
Belgium	Biosafety Advisory Council	1. General comments	Comment from Belgium	We do not have any comments and we consider all the necessary information is present to conduct a robust risk assessment.	The GMO Panel thanks for the Comment.
France	Ministry of agriculture	1. General comments	Comment 1	Main comments: The HCB Scientific Committee recognises that the new information supplied by the applicant does not provide evidence of any new risks to the environment. It nevertheless notes the following: The updated gene-transfer risk assessment is	The applicant submitted updated bioinformatic HGT analysis using up-to-date databases (spontaneous information 16/04/2020). The analysis was performed according to EFSA guideline (EFSA, 2017). Given the results of this analysis and that the recombinant DNA in maize Bt11 does not confer selective advantages to

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				confusing in that the main text of the renewal application is at variance on this subject with the relevant part of the updated annex, which identifies regions of homology capable in theory of contributing to homologous recombination events that could result in transfer of the cry1Ab expression cassette to bacteria. However, the HCB Scientific Committee agrees with the applicant's conclusions, considering that if these unlikely events were to occur, the attendant environmental and health consequences would be negligible; Occurrence of teosinte populations compatible with cultivated maize was reported in the European Union during the marketing period. The risk from potential gene transfer from Bt11 maize to teosinte ought now to be considered by the applicant; Lastly, in general, both the original application and the renewal application refer only to import into temperate regions of the European Union. Yet the European Union also includes outermost regions in tropical zones more conducive to persistence of maize. This is the case for some French overseas departments and regions. The HCB Scientific Committee would like the specific environmental characteristics of these regions to be taken into consideration in safety assessment and monitoring plans for applications for placing on the market of seed from genetically modified plants in the European Union. One alternative	microorganisms, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT. The information/datasets provided by the applicant for the renewal of authorisation of maize Bt11 are in line with the requirements outlined in the EFSA guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003 (EFSA, 2015). In its scientific opinion on application EFSA-GMO-RX-016, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize Bt11.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				would be to exclude these regions from the marketing area. Additional comments: Some members of the HCB Scientific Committee have emphasised that a broader study of the consequences for Europe of cultivation of Bt11 maize in exporting third-countries would be desirable, not only in socio-economic terms but also concerning biodiversity. They point out that, under the Convention on Biological Diversity, exporting countries have international responsibilities with regard to threatened species. They suggest that the application should mention the results of an assessment of the crop's biodiversity impact in producing and exporting countries. In addition, they recommend taking account of how import of this maize influences selection of crops in Europe and therefore the biodiversity resulting from these agrosystem choices. Lastly, some members of the HCB Scientific Committee have raised the ethical issue of authorising import into the European Union of a commodity whose production in the exporting countries will entail operators' exposure to a plant protection product (containing glufosinate-ammonium) that has been withdrawn from the French market on health grounds.	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
France	Ministry of agriculture	2.2. Post-market monitoring and post-market environmental monitoring reports	Comment 2	The figures given by Syngenta in its annual post-market monitoring reports for import of maize grain (GM and non-GM) by EU Member State and by country of origin raise questions, since the figures given for France do not tally with the figures obtained from local official sources, whether for the quantities imported or for their origin. Moreover, depending on the year, the comments on the tables for import of maize in tons are out by a factor of 1,000. The HCB Scientific Committee is surprised that the latest monitoring reports do not mention the occurrence of teosinte populations compatible with cultivated maize, recently reported in Spain (1) (Pardo et al., 2014; Pardo et al., 2015) and France (2) (Arvalis, 2013), in terms of the attendant risk of potential gene transfer from GM maize to teosinte (Devos et al., 2018; EFSA, 2016; HCB, 2016; Trtikova et al., 2017). 1. Centro de Sanidad y Certificacíon Vegetal, Gobierno de Aragon, Informaciones fitosanitarias, septiembre 2014, https://www.aragon.es/estaticos/GobiernoAragon/Departamentos/AgriculturaGanaderiaMedioAmbiente/TEMAS_AGRICULTURA_GANADERIA/Areas/03_Sanidad_Vegetal/PUBLICACIONES_CSCV/I_F_TEOSINTE.pdf. 2. Arvalis, 2013; Deux-Sèvres Chamber of Agriculture, http://www.agri79.com/actualites/teosinte-la-teosinte-exige-une-vigilance-toute-	The GMO Panel took note of the comment.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				particuliere:JFNK3KKU.html Arvalis (2013). Téosinte : une adventice qui demande une vigilance toute particulière. In: Information technique du Service Communication Marketing Arvalis, Institut du végétal. Paris, 1-4. Devos, Y., Ortiz-Garcia, S., Hokanson, K.E., and Raybould, A. (2018). Teosinte and maize x teosinte hybrid plants in Europe — Environmental risk assessment and management implications for genetically modified maize. Agric Ecosyst Environ 259, 19-27. EFSA (2016). Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094. HCB (2016). Avis HCB-2016.03.17 du Comité scientifique du HCB relatif à une demande d'autorisation de mise sur le marché du maïs génétiquement modifié 1507x59122 à des fins de culture, d'importation, de transformation et d'alimentation humaine et animale (dossier EFSA-GMO-NL-2005-28). Available at: http://www.hautconseildesbiotechnologies.fr. (Paris), pp. 73. Pardo, G., Cirujeda, A., Betrán, E., Fernández-Cavada, S., Fuertes, S., Rodríguez, E., Perdiguer, A., Aibar, J., and Zaragoza, C. (2014). El Teosinte (Zea	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				mays, spp.) In Informaciones Técnicas GIoberno del Aragone (Dirección General De Alimentación Y Fomento Agroalimentario, Centro de Sanidad y Certificacion Vegetal), pp. 6. Pardo, G., Fuertes, S., Fernández-Cavada, S., Betrán, E., Cirujeda, A., Marí, A.I., Aibar, J., Zaragoza, C., Perdiguer, A., Llenes, J.M. et al. (2015). Presencia de teosinte (Zea spp.) como mala hierba en los regadíos del valle del Ebro. In XV Congreso de la Sociedad Española de Malherbología: La Malherbología y la transferencia tecnológica, Junta de Andalucia ed, (Sevilla, 19 - 22 octubre 2015), pp. 417-423. Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., and Hilbeck, A. (2017). Teosinte in Europe — Searching for the origin of a novel weed. Scientific Reports 7.	
France	Ministry of agriculture	2.3.1. Systematic search and evaluation of literature	Comment 3	A review of scientific literature and new data relating to Bt11 maize has been conducted, covering a period of 10 years (January 2008 to September 2018). The Medline, Agricola, CAB Abstracts, Biosis, Toxcenter, FSTA, Frosti, Polluab, AquaSci, Biotechno, Esbiobase, Bioeng, Aqualine and Embase databases were searched. According to the application, searches were made on terms linked to Bt11, Cry1Ab and PAT. In all, 2,029 papers were retrieved from the databases, and over a hundred were studied in depth. No papers showed any new environmental or health risks. The HCB Scientific Committee notes that insect-resistance search terms have not been used since	In addition to the literature searches submitted in the context of the annual post-market environmental monitoring reports, the applicant submitted a systematic literature search and two updated literature searches covering a period of The GMO Panel assessed the applicant's literature searches on maize Bt11 according to the guidelines given in EFSA (2010, 2017). EFSA identified some points requiring further clarification. The applicant was requested to: (i) explain why truncation was not applied consistently in all variants used for the Cry1Ab protein, and assess the impact on the retrieval performance and search outcomes; (ii) clarify whether there was a typo in the term "phosphino thricinthricin", and assess the impact on the retrieval

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				2011. Moreover, target organisms do not seem to have been included in the criteria for the literature review. The HCB Scientific Committee has doubts regarding the applicant's coverage of papers on insect resistance to the Cry1Ab toxin.	performance and search outcomes; (iii) clarify whether the search strategies were adapted to search the specific subject headings offered by the individual databases searched. The applicant provided the requested additional information on 20 June 2019. The overall quality of the performed literature search was acceptable.

France	Ministry of agriculture	2.3.2. Updated bioinformatics	Comment 4	Analysis of flanking regions The new analyses of flanking regions, dating from March 2018, are still confined to 352 bp of the 5' upstream and 540 bp of the 3' downstream genomic sequences flanking the insert. The HCB Scientific Committee notes that the applicant has not widened sequencing to cover 1,000 bp for each of these regions, as recommended by EFSA in order clearly to determine whether endogenous genes have been interrupted (EFSA, 2015). The analyses using the most recent NCBI databases have confirmed the presence of repetitive maize sequences on either side of the insertion site that could be knob or repetitive heterochromatin sequences (Appendices 2.3.2-01_BLASTN_Bt11; 2.3.2-02_BLASTN_Bt11; 2.3.2-03_BLASTX_Bt11). None of the alignments found showed homology with known maize coding or regulatory sequences at the insertion site. On balance, the HCB Scientific Committee believes that this analysis is enough to rule out disruption of maize regulatory sequences or genes by the insert Analysis of horizontal gene transfer.	The applicant submitted updated bioinformatic analysis using up-to-date databases to identify any interrupted maize endogenous genes (spontaneous information 16/04/2020). The update bioinformatic analysis was performed using NCBI non-redundant nucleotide (nr/nt) database, non-redundant protein (nr) database, and EST database. The analysis confirmed indeed that no endogenous maize regulatory sequence or gene has been interrupted. The applicant submitted updated bioinformatic HGT analysis using up-to-date databases (spontaneous information 16/04/2020). The analysis was performed according to EFSA guideline (EFSA, 2017). Given the results of this analysis and that the recombinant DNA in maize Bt11 does not confer selective advantages to microorganisms, the GMO Panel agrees with HCB comment that no safety concern is linked to an unlikely but theoretically possible HGT. The GMO Panel thanks HCB for the comment. The alignment showing identity to a CT43 *Bacillus thuringiensis* plasmid does not meet the requirement of 'at least two regions of similarity between the Bt11 insert and the source of the microbial sequence'. For this reason, the hit was not further investigated by the applicant. The GMO Panel thanks HCB for the comment.

					The GMO Panel takes note of the HCB comment regarding the applicability of the EFSA guidance on HGT analysis (EFSA, 2017).

Country	Organization	Reference	Topic	Comment	GMO Panel responses
France	Ministry of agriculture	(2.3.2. Updated bioinformatics)	(Comment 4)	The applicant has carried out an assessment to determine the possibility of homologous recombination between the insert and microbial DNA sequences in line with EFSA guidance, i.e. to show 95% identity over a sequence of at least 200 bp in length and the presence of at least two regions of similarity between the Bt11 insert and a particular microbial sequence. Screening was carried out for homology between the insert sequence and DNA sequences in the NCBI databases (December 2017) for microbial genomes, plasmids and bacteriophages (Appendix 2.3.2-08_BLASTN microbial_Bt11). According to Appendix 2.3.2-08_BLASTN microbial_Bt11, the search results from the NCBI Complete Microbial Genomes database show homology meeting the EFSA criteria for two accession numbers, one for Mycoplasma mycoides sbsp. capri str. GM12 and the other for Bacillus subtilis BSn5. In the NCBI Representative Plasmids database, alignments were found with Escherichia coli (four accession numbers), Bacillus cereus (one accession number) and Staphylococcus aureus (two accession numbers). This is at variance with the application's main text, where it is stated (Part II Scientific info, Main Text_EFSA-GMO-RX002, p. 17): 'The results of these analyses returned no alignments that met the threshold of 95% identity in alignments of at least 200 base pairs in length	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				and at least two regions of similarity between the Bt11 insert and a microbial sequence.' However, there is a more detailed analysis in Appendix 2.3.2-08_BLASTN microbial_Bt11. For each of these accession numbers, the regions of homology identified correspond to pUC18 plasmid sequences on either side of the cry1Ab expression cassette (comprising the 35S promoter, the cry1Ab gene and the NOS terminator). The applicant has assessed the probability of horizontal transfer of the cry1Ab expression cassette to these bacteria by double homologous recombination. Since the distance separating the two regions of homology with sequences from each of these accession numbers is over 3,100 bp in the Bt11 insert, the applicant considers, on the basis of bibliographical data (Kung et al., 2013), that double recombination is unlikely (the HCB Scientific Committee notes that the paper cited concerns recombination efficiency in Xylella fastidiosa and that the data reported cannot be extrapolated to all other bacteria). Even if the applicant considers transfer of the cry1Ab expression cassette to bacteria by double homologous recombination to be unlikely, its consequences have nevertheless been examined in the application. This gene could be expected to have a limited level of expression because of codon optimisation according to preferred usage for maize and because of the presence of a 35S	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				plant virus promoter and an intron, which are not suited to expression in a prokaryotic system. Furthermore, since the cry1Ab gene comes from a bacterium belonging to the genus Bacillus, naturally present in the environment, its hypothetical transfer would at most result in an insignificant increase in environmental exposure to the toxin without constituting a new environmental risk. The HCB Scientific Committee agrees with the applicant's conclusions. The HCB Scientific Committee questions why the applicant's bioinformatic analysis has identified no homologies with cry1Ab gene sequences from Bacillus thuringiensis ssp. kurstaki HD-1 and with the pat gene sequences from Streptomyces viridochromogenes inserted in the construct. It is possible that codon optimisation according to preferred usage for maize could reduce identity levels below the applicant's 95% threshold for 200 bp lengths. Could the applicant provide more information on these alignments? Lastly, Appendix 2.3.2-08_BLASTN microbial_Bt11 reports, amongst others, alignments having 98% identity to a CT43 Bacillus thuringiensis plasmid over 290 bp. Could the applicant explain why these alignments are not taken into consideration in analysis of homology with the insert? In any case, if transfer of the cry1Ab and pat transgenes did occur, in Bacillus thuringiensis and	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Streptomyces viridochromogenes respectively, the recombination mechanism involved would lead to replacement of a functional allele from the indigenous bacterium by an allele possibly associated with the construct promoter, whose expression in a prokaryotic organism would be weak or even nil, thus rendering the allele non-functional. Even if it was functional, the consequences would be neutral. Lastly, transfer to other bacteria by illegitimate recombination, an even rarer event, would encounter the same expression limits as above, and even if expression were to be significant, the consequences for the taxonomic structure of the relevant ecosystem microbiota and their functioning would be negligible. More broadly, concerning the EFSA guidance on sequence similarity searches for assessment of horizontal gene transfer from plants to microorganisms, the HCB Scientific Committee would like to make the following comments: While it is true that horizontal transfer is facilitated by (1) the possibility of double homologous recombination, (2) near-perfect similarity over homologous regions flanking the relevant fragment, (3) homologous regions of a length suitable for recombination in recipient bacteria, and (4) a distance between these homologous regions that is suited to recipient bacteria (there is no general rule), the fact	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				remains that horizontal transfer is possible (1) with a single region of homology, either through single homologous recombination (and addition of a sequence instead of a sequence replacement in the recipient genome) or through double homologous recombination over a region of homology long enough for recombination to occur at both ends, and (2) with a lower degree of similarity for what are known as homeologous recombinations, or even illegitimate recombinations (Meier and Wackernagel, 2003), even though the likelihood of transfer diminishes with the degree of similarity, although it is not clear whether there is a threshold beneath which recombination becomes impossible, and bearing in mind that the impact of any mismatches will differ according to their distribution in the region of homology. It is further worth noting that the likelihood of horizontal transfer also varies depending on the relevant genomic regions of the bacteria and the extent to which they facilitate recombination (Fall et al., 2007; Ray et al., 2009). Thus, the threshold of 95% identity for an alignment of at least 200 bp and the repetition of a region of homology, as recommended by EFSA, appear arbitrary and too basic for drawing clear-cut conclusions one way or the other regarding the possibility of gene transfer. Even though it considers the different factors that could facilitate transfer to microorganisms of a	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				bacterial sequence in a GM plant gene expression cassette, the HCB Scientific Committee systematically examines the consequences that such a transfer might have if it were to occur. In field cultivation, given the volume of soil concerned and the fact that there are some 1 billion bacteria per gramme of soil, even the most unlikely event cannot be ruled out. EFSA (2015). EFSA GMO Panel guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003. EFSA Journal 13(6):4129, 8 pp. Fall, S., Mercier, A., Bertolla, F., Calteau, A., Gueguen, L., Perriere, G., Vogel, T.M., and Simonet, P. (2007). Horizontal gene transfer regulation in bacteria as a "Spandrel'' of DNA repair mechanisms. Plos One 2. Kung, S.H., Retchless, A.C., Kwan, J.Y., and Almeida, R.P.P. (2013). Effects of DNA size on transformation and recombination efficiencies in Xylella fastidiosa. Appl Environ Microbiol 79, 1712-1717. Meier, P., and Wackernagel, W. (2003). Mechanisms of homology-facilitated illegitimate recombination for foreign DNA acquisition in transformable Pseudomonas stutzeri. Mol Microbiol 48, 1107-1118. Ray, J.L., Harms, K., Wikmark, O.G., Starikova, I., Johnsen, P.J., and Nielsen, K.M. (2009). Sexual	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				isolation in Acinetobacter baylyi is locus-specific and varies 10,000-fold over the genome. Genetics 182, 1165-1181.	
France	Ministry of agriculture	2.3.3. Additional documents or studies performed by or on behalf of the applicant	Comment 5	On 18 October 2018, the applicant sent EFSA a new document reporting fresh experimental data for molecular characterisation, including, amongst other things, verification of the Bt11 insert sequence. Document 2.3.1.-1_Annex 2_List of Studies -Bt11_new states on pp. 4/5, in a report on a 2012 study: 'The DNA sequence analysis demonstrated that the Bt11 insert was intact, there were no nucleotide differences between the insert and the transformation fragment, and the organization of the genetic elements within the insert, as present in plasmid pZO1502, was maintained. The NotI restriction sites used to create the transformation fragment, and two additional base pairs (bp), adjacent to the NotI restriction site, were not transferred into Bt11 maize.' This paragraph seems self-contradictory, first stating that the insert was intact and then that it was not, compared to the DNA fragment used for the transformation, since the NotI sites were deleted together with two additional base pairs at one end of the NotI fragment. While the HCB Scientific Committee considers that these modifications ought not to affect the cry1Ab	The applicant has clarified in the dossier that the sequence of the event Bt11 is identical to the sequence of the event originally assessed. The GMO Panel performed the assessment under this assumption. The GMO Panel thanks HCB for the assessment and takes note of the comment.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				and pat gene expression cassettes, it would nevertheless like the applicant to report the data and formulate its conclusions more meticulously.	
France	Ministry of agriculture	4. Monitoring plan and proposal for improving the conditions of the original authorisation	Comment 6	Since no adverse effects have been reported during the ten years of Bt11 maize marketing in the European Union, no changes to the original monitoring plan have been proposed (other than updating the format). The post-market monitoring plan meets regulatory requirements. The HCB Scientific Committee agrees with the general surveillance approach proposed by the applicant. However, it advises the applicant to contact the various local operators handling Bt11 maize in order to call their attention to appropriate steps for preventing or limiting accidental release: sheeting of haulage lorries, subsequent inspection of routes used by haulage units between place of import and storage/processing sites for possible mechanical or chemical treatment (other than with glufosinate-ammonium) of road verges in the event of spillage. Monitoring measures should be adapted to the specific context in each Member State, taking particular account of regions where teosinte populations have been reported. After ten years of post-market monitoring of Bt11 maize without any adverse effects having been reported, some experts have asked whether the resources committed to monitoring are commensurate with the risks involved, whether it is appropriate to maintain this level of monitoring	In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				for the next ten years of marketing and whether it might be advisable to review current regulations.	
Germany	BfN	1. General comments	Comment 1 / Federal Agency for Nature Conservation (BfN)	The Federal Agency for Nature Conservation (BfN) suggests that the environmental risk assessment of Bt11 maize should stronger make use of scientific information after the last authorization. Areas identified are: i) fate of Bt toxins in the environment after livestock has been fed with Bt maize; ii) occurrence of teosinte as a wild relative of maize in Europe. Further analysis should be presented before the application can be renewed. In addition, the monitoring plan based on consent given by Commission Decision 2010/419/EU and the monitoring reports (2009 to 2017) have many deficiencies and are neither in line with Directive 2001/18/EC and the corresponding guidelines nor with EFSA guidance on the post-market environmental monitoring (PMEM) of genetically modified plants (EFSA 2011). Therefore, the applicant is asked to complete the monitoring reports and to revise and to detail the monitoring plan (see 2.2 and 4. for details). An improved monitoring plan and completed monitoring reports have to be provided before consent for renewal may be given (EFSA 2015, Section 4.) References: EFSA (2011). Scientific opinion. Guidance on the Post-Market Environmental monitoring (PMEM) of genetically modified plants The EFSA Journal, 9(8): 2316, 40 pp. EFSA (2015). Guidance for renewal applications of	The GMO Panel took note of these comments. The information/datasets provided by the applicant for the renewal of authorisation of maize Bt11 are in line with the requirements outlined in the EFSA guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003 (EFSA, 2015). In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				genetically modified food and feed authorised under regulation (EC) No 1829/2003 The EFSA Journal 13(6):4129 1-8.	
Germany	BfN	2.2. Post-market monitoring and post-market environmental monitoring reports	Comment 2 / Federal Agency for Nature Conservation (BfN)	Case-specific monitoring BfN is of the opinion that a case-specific monitoring addressing loss and spillage during transport, storage and handling in the environment as well as the efficacy of management measures applied is necessary. General surveillance The general surveillance plan is very unspecific concerning the monitoring methodology applied. Furthermore, detailed information about third parties involved in the monitoring is missing. The monitoring reports also do not comprise any detailed information concerning these topics. Monitoring reports (2009-2017) The monitoring reports of Bt11 maize for the authorization period have severe shortcomings. They do not provide sufficient sound data to support the conclusion that no adverse health or environmental effects associated with the import and use of the GMO have appeared. Com-pleted and detailed monitoring reports are needed to be able to draw conclusions based on monitoring data.	As no potential adverse environmental effects were identified in the environmental risk assessment (ERA) of maize Bt11 (EFSA, 2009), case-specific monitoring was not considered necessary by the GMO Panel. Moreover, in its scientific opinion on application EFSA-GMO-RX-016, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize Bt11. In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further

Country	Organization	Reference	Topic	Comment	GMO Panel responses
					discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).
Germany	BfN	2.3.1. Systematic search and evaluation of literature	Comment 3 / Federal Agency for Nature Conservation (BfN)	With regard to information related to food and feed the applicant should include the following references (missing in the folder '2.3.1-01_Review Literat-Studies_Bt11') which refer to either Bt11, Cry1Ab or useable methods for the assessment: References: Bednarek D, Dudek K, Kwiatek K, tkiewicz Sawh, Strzetelski J (2013) Effect of a diet composed of genetically modified feed components on the selected immune parameters in pigs, cattle, and poultry. Bulletin of the Veterinary Institute in Pulawy 57 (2): 209–217. Bondzio A, Stumpff F, Schön J, al e (2008) Impact of Bacillus thuringiensis toxin Cry1Ab on rumen epithelial cells (REC) - A new in vitro model for safety assessment of recombinant fodd compounds. Food and Chemical Toxicology 46: 1976–1984. Buzoianu SG, Walsh MC, Rea MC, Cassidy JP, Ryan TP et al. (2013) Transgenerational effects of feeding genetically modified maize to nulliparous sows and offspring on offspring growth and health. Journal of Animal Science 91 (1): 318–330. Buzoianu SG, Walsh MC, Rea MC, O'Donovan O, Gelencsér E et al. (2012) Effects of feeding Bt maize to sows during gestation and lactation on	The GMO Panel took note of the comments. Following the requirements of the EFSA guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015), the applicant performed a literature search in the context of application EFSA-GMO-RX-016. The literature search was performed in accordance to the recommendations outlined in the EFSA explanatory note for literature searching (EFSA, 2017) and was considered acceptable by the GMO Panel. Details on the outcome of the literature search can be found in the corresponding section of the EFSA GMO Panel scientific opinion. The GMO Panel acknowledged that no scientific publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on maize Bt11 had been identified by the applicant.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				maternal and offspring immunity and fate of transgenic material. PLoS ONE 7 (10): 47851. Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Cotter PD et al. (2012) High-throughput sequence-based analysis of the intestinal microbiota of weanling pigs fed genetically modified MON810 maize expressing *Bacillus thuringiensis* Cry1Ab (Bt maize) for 31 days. Applied and Environmental Microbiology 78 (12): 4217–4224. Gu J, Krogdahl A, Sissener NH, Kortner TM, Gelencser E et al. (2013) Effects of oral Bt-maize (MON810) exposure on growth and health parameters in normal and sensitised Atlantic salmon, Salmo salar L. British Journal of Nutrition 109: 1408–1423. Gu J, Bakke AM, Valen EC, Lein I, Krogdahl (2014) Bt-maize (MON810) and non-GM soybean meal in diets for Atlantic salmon (Salmo salar L.) juveniles - impact on survival, growth performance, development, digestive function, and transcriptional expression of intestinal immune and stress responses. PLoS ONE 9 (6): 99932. Halle I, Flachowsky G (2014) A four-generation feeding study with genetically modified (Bt) maize in laying hens. Journal of Animal and Feed Sciences 23 (58): 63. Haller S, Romeis J, Meissle M (2017) Effects of purified or plant-produced Cry proteins on *Drosophila melanogaster* (Diptera: Drosophilidae) larvae. Scientific Reports 7 (1): 11172. Kadlec J, Rehout V, ítek J, Hanusová L, Hosnedlová	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				B (2009) The influence of GM Bt maize MON 810 and RR soya in feed mixtures upon slaughter, haematological and biochemical indicators of broiler chickens. Journal of Agrobiology 26 (1): 51–55. Mao J, Sun X, Cheng J, Shi Y, Wang X et al. (2016) A 52-week safety study in cynomolgus macaques for genetically modified rice expressing Cry1Ab/1Ac protein. Food and Chemical Toxicology 95: 1–11. Rodriguez-Nogales JM, Cifuentes A, Garcia MC, Marina ML (2010) Improved methodology for the characterisation of transgenic Bt-11 maize cultivars using RP-HPLC profiles of albumin, globulin, prolamin, and glutelin protein fractions and chemometric analysis. Food Chemistry 120 (4): 1229–1237. Swiatkiewicz S, Koreleski J, Arczewska-Wlosek A, Swiatkiewicz M, Twardowska M et al. (2011) Detection of transgenic DNA from Bt maize and herbicide tolerant soybean meal in tissues, eggs and digestive tract content of laying hens fed diets containing genetically modified plants. Annals of Animal Science 11 (3): 413–424. Zdziarski IM, Carman JA, Edwards JW (2018) Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. FNS 09 (06): 763–796. Missing literature referring to the environmental risk assessment are listed under 3.2 B).	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
Germany	BfN	2.3.2. Updated bioinformatics	Comment 4 / Federal Agency for Nature Conservation (BfN)	Disruption of endogenous genes According to the applicant no endogenous maize genes are disrupted at the insertion site of event Bt11 (CBI: From Report Number: SSB-110-18 and SSB-112-18). The updated bioinformatics are based on BLAST analyses of 540 bp and 352 bp, respectively, flanking the insertion site. According to the EFSA guidance for renewal applications (EFSA 2015b) "…a sequencing length of 1 kb on each side of the insert(s) is recommended for the characterization of flanking sequences, in case the originally determined flanking regions did not allow to clearly determine whether known endogenous genes were interrupted…". Since the sequence information provided by the applicant does not allow locating the site of insertion of event Bt11 in the maize genome, the applicant should provide sufficiently long sequence data covering the insertion site to assure that it is not located within the intron region of a functional gene.	The applicant is not requested to provide new sequencing data in the frame of renewal applications. The applicant clarified that the sequence of the event Bt11 is identical to the sequence of the event originally assessed. The applicant submitted updated bioinformatic analysis using up-to-date databases to identify any interrupted maize endogenous genes (spontaneous information 16/04/2020). The update bioinformatic analysis was performed using NCBI non-redundant nucleotide (nr/nt) database, non-redundant protein (nr) database, and EST database. The analysis confirmed indeed that no endogenous maize regulatory sequence or gene has been interrupted.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
Germany	BfN	3. Overall assessment	Comment 5 / Federal Agency for Nature Conservation (BfN)	Interplay between environmental risk assessment and monitoring The information necessary to conclude on the ERA is still partly missing. Thus, the safety of Bt11 maize cannot be fully assessed. Depending on those results the conclusions concerning case-specific monitoring may need to be revised. Environmental risk assessment A) Teosinte Teosinte has been reported to occur in Spain and France (EFSA 2016). As GM maize is mainly imported to Spain (see monitoring reports 2008 to 2016) gene flow from GM maize to teosinte and vice versa must be considered in the risk assessment and monitoring. In the context of applications for import and processing of GM maize the occurrence of volunteer and feral maize plants in Europe (Pascher 2016) need to be included in the assessment of potential gene flow between GM maize and teosinte. The potential for gene flow between teosinte and cultivated maize is high, especially for Zea mays ssp. parviglumis, for which hybridization rates of 50% and more have been reported (Ellstrand et al. 2007, Chavez et al. 2012). Chavez et al. concluded that biosafety regulators in regions where teosinte occurs should not only consider outcrossing from maize to teosinte but also the possibility of teosinte acting as a genetic bridge back to maize. Teosinte is difficult to control and is considered an	The GMO Panel took note of the comments. Following the requirements of the EFSA guidance for renewal applications of genetically modified food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015), the applicant performed a literature search in the context of application EFSA-GMO-RX-016. The literature search was performed in accordance to the recommendations outlined in the EFSA explanatory note for literature searching (EFSA, 2017) and was considered acceptable by the GMO Panel. Details on the outcome of the literature search can be found in the corresponding section of the EFSA GMO Panel scientific opinion. The GMO Panel acknowledged that no scientific publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on maize Bt11 had been identified by the applicant. As no potential adverse environmental effects were identified in the environmental risk assessment (ERA) of maize Bt11 (EFSA, 2009), case-specific monitoring was not considered necessary by the GMO Panel. Moreover, in its scientific opinion on application EFSA-GMO-RX-016, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize Bt11. Given that environmental exposure of nontarget organisms to spilled GM seeds or occasional feral GM maize plants arising from spilled GM seeds is limited, and because most proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the GM plant with non-

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				agricultural pest which needs management. Teosinte flowers earlier and longer than maize and pollen of both species can spread over long distances. The kernels can remain for long periods in the seed bank. Recent evidence confirms the occurrence of hybrids between maize and teosinte in Europe and the existence of a new teosinte subspecies in Europe (Tritikova et al. 2017). References: Chavez, N. B., Flores, J. J., Martin, J., Ellstrand, N. C., Guadagnuolo, R., Heredia, S., & Welles, S. R. (2012). Maize x teosinte hybrid cobs do not prevent crop gene introgression. Economic botany, 66(2), 132-137. EFSA (2016). Relevance of new scientific evidence on the occurrence of teosinte in maize fields in Spain and France for previous environmental risk assessment conclusions and risk management recommendations on the cultivation of maize events MON810, Bt11, 1507 and GA21. EFSA supporting publication 2016:EN-1094. 13 pp. Ellstrand, N. C., Garner, L. C., Hegde, S., Guadagnuolo, R., & Blancas, L. (2007). Spontaneous hybridization between maize and teosinte. Journal of Heredity, 98(2), 183-187. 2007. Pascher, K. (2016). Spread of volunteer and feral maize plants in Central Europe: Recent data from Austria. Environmental Sciences Europe, 28 (1), 30. Trtikova, M., Lohn, A., Binimelis, R., Chapela, I.,	target organisms are not considered a relevant issue by the GMO Panel.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Oehen, B., Zemp, N., Widmer, A. & Hilbeck, A. (2017). Teosinte in Europe - Searching for the Origin of a Novel Weed. Scientific Reports, 7 (1), 1560. B) Fate of Bt Proteins in the Environment For Bt proteins, in principle, the exposure route from feed, via manure into the environment has been demonstrated (e.g. Gruber et al. 2011; Gürtler et al. 2010, Paul et al. 2010). To our understanding present studies are not sufficient to conclude that exposure of the environment and thus effects on non-target organisms will be negligible. Instead, further experiments are necessary to conclude on the exposure and subsequent effects and risks for non-target organisms from the exposure to Bt proteins via manure or sewage. References which should be used to assist the assessment and have not been included into the results of the literature search by the applicant (folder '2.3.1-01_Review Literat-Studies_Bt11'): Bai Y, Yan R, Ke X, Ye G, Huang FN et al. (2011) Effects of transgenic Bt rice on growth, reproduction, and superoxide dismutase activity of Folsomia candida (Collembola. Isotomidae) in laboratory studies. J Econ Ent 104 (6): 1892–1899. Bakonyi G, Dolezsai A, Mátrai N, Székács A (2011) Effects of consumption of Bt-maize (MON 810) on the collembolan *Folsomia candida*, over multiple generations. A laboratory study. Insects 2 (2):	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				243–252. Campos RC, Holderbaum DF, Nodari RO, Hernandez MIM (2018) Indirect exposure to Bt maize through pig faeces causes behavioural changes in dung beetles. J. Appl. Entomol. 57 (3): 117. Gürtler P, Brandl C, Meyer HHD, Tichopad A (2012) Feeding genetically modified maize (MON810), to dairy cows. Comparison of gene expression pattern of markers for apoptosis, inflammation and cell cycle. Journal für Verbraucherschutz und Lebensmittelsicherheit 7: 195–202. Gürtler SP (2015) Long-term feeding of genetically modified maize (MON810) - Metabolism of recombinant DNA and the novel protein in the dairy cow. Dissertation Technische Universität München (TUM), 1-93 p. Hilbeck, A. & Otto, M. (2015) Specificity and Combinatorial Effects of Bacillus Thuringiensis Cry Toxins in the Context of GMO Environmental Risk Assessment. Frontiers in Environmental Science, 3, 71. Jänsch, S., Bauer, J., Leube, D., Otto, M., Römbke, J., Teichmann, H. & Waszak, K. (2018) A new ecotoxicological test method for genetically modified plants and other stressors in soil with the black fungus gnat Bradysia impatiens (Diptera): current status of test development and dietary effects of azadirachtin on larval development and emergence rate. Environmental	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				Sciences Europe, 30 (1), 654. Mueting SA, Strain KE, Lydy MJ (2014) Validation of an extraction method for Cry1Ab protein from soil. Environmental Toxicology and Chemistry 33 (1): 18–25. Sharma P, Nain V, Lakhanpaul S, Kumar PA (2010) Synergistic activity between Bacillus thuringiensis Cry1Ab and Cry1Ac toxins against maize stem borer (Chilo partellus Swinhoe). Letters in Applied Microbiology 51 (1): 42–47. Székács A, Weiss G, Quist D, Takács E, Darvas B et al. (2012) Inter-laboratory comparison of Cry1Ab toxin quantification in MON 810 maize by enzyme-immunoassay. Food and Agricultural Immunology 23 (12): 99–121. Takács E, Nagy A, Gelencsér +, Székács A (2015) Internal Quality Control of an enzyme-linked immunoassay for Cry1Ab toxin detection applied in animal tissues. Acta Alimentaria (Budapest) 44 (4): 593–600. van der Merwe F, Bezuidenhout C, van den Berg J, Maboeta M (2012) Effects of Cry1Ab transgenic maize on lifecycle and biomarker responses of the earthworm, Eisenia andrei. Sensors 12 (12): 17155–17167.	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
Germany	BfN	4. Monitoring plan and proposal for improving the conditions of the original authorisation	Comment 6 / Federal Agency for Nature Conservation (BfN)	General Monitoring the environmental effects of Bt11 maize should serve as an early warning system: The data which will be collected should be relevant to and suitable for a "rapid assessment and implementation of measures to reduce any consequences to the environment" (Council Decision 2002/811/EC). To achieve this aim a meaningful monitoring plan in line with the scope of use of the GMO has to be provided. The plan has to meet the following scientifically recognised minimum standards: - A fully specified list of monitoring parameters has to be provided. - An operating schedule giving full details of points in time is requested. - The methods of data analysis including the statistical methods have to be elaborated. - The baseline status of the receiving environment has to be characterised. - The applicant is requested to indicate how the monitoring plan is adapted to various local conditions. - The time-period of monitoring needs to be sufficient to detect delayed, long-term or cumulative adverse effects and check of requirements of certain monitoring parameter. Furthermore, the monitoring should be run primarily in regions, where the GMO will be transported, stored, packaged, processed or used. Since traders may commingle the GMO with other	In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems). As no potential adverse environmental effects were identified in the environmental risk assessment (ERA) of maize Bt11 (EFSA, 2009), case-specific monitoring was not considered necessary by the GMO Panel. Moreover, in its scientific opinion on application EFSA-GMO-RX-016, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				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 the GMO and those caused by other GM maize. Case-specific monitoring The case-specific monitoring has to focus on pathways where Bt11 maize or material containing the GMO get into the environment, which occurs during transport, processing or use of the GMO as food and feed. The applicant is requested to provide an appropriate case-specific monitoring plan, comprising the following elements at least: i) spillage or loss of the GMO during transport, storage, packaging, processing and use (food and feed), ii) potential spread and persistence of the GMO, if spillage or loss of viable grains of the GMO occurs, iii) if spread and persistence of the Cry1 Ab protein occur, further observations of impacts on organisms, food chains, and habitats are required, iv) exposure of the Cry1 Ab protein to the environment e.g. via sewage water, waste material or by-products which occur during processing or use of the GMO material as food/feed, v) the GMO may enter the environment together with other approved GM maize lines containing	the conclusions of the original risk assessment on maize Bt11.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				different Cry proteins. Therefore, a special focus should be on combined effects, vi) occurrence of teosinte species in regions affected by transport, storage, packaging, processing and use (feed and food) and subsequently potential outcrossing of the transgenes. For these parameters, the use of the following methods is recommended (www.vdi.eu/vdi-standards): • VDI-Standard 4330 Part 10 “Floristic mapping of genetically modified plants, their crossing partners and their hybrid offspring”, • VDI-Standard 4330 Part 5 “Guideline for the collection and preparation of plant samples for molecular biological analysis”. The BfN is of the opinion that risk management measures like the control of adventitious maize plants and clean-up measures to control viable plant material during transport, storage, packaging or processing should be confirmed as mandatory. The efficacy of the implemented risk management measures should be monitored during case-specific monitoring (EFSA 2011). General surveillance for unanticipated adverse effects The general surveillance plan needs further specification concerning the monitoring method-ology to be applied (parameters, methods, locations etc.).	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				The general surveillance plan has to focus on possible pathways how Bt11 maize can enter the broader environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal and use of the GMO. The applicant has to provide an appropriate general surveillance plan comprising the monitoring of spillage or losses of the GMO during transport, storage, packaging, processing and use as well as potential spread and persistence and on possible combined effects with other approved GM maize. The general surveillance plan provided is predominantly based on collaboration with third parties. Therefore, the monitoring expertise of external people involved in the monitoring activities and detailed information about participating networks (e.g. name, EU country, responsible authority, availability, scope of monitoring, composition of the network) have to be specified. Binding agreements/contracts with third parties (external people and existing net-works) are requested which clearly determine what data are provided and how these data are made available. The professional qualification of involved people and the involvement of other operators further down the food chain should be specified. This information is so far neither part of the monitoring plan nor of the monitoring reports. Reporting the results of the monitoring The applicant is required to report on the	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				monitoring methodology applied (parameters, methods, locations, involved stakeholders etc.) and on the results of the monitoring. All raw data have to be made available. Moreover, the monitoring report should also deliver detailed information on i) actual volumes of Bt11 maize imported into the EU (separated information from conventional maize), ii) the ports and silos where shipments of the GMO were unloaded, iii) the processing plants where the GMO was transferred to, iv) the amount of the GMO used on farms for feed, and v) transport routes of the GMO. References: EFSA (2011). Scientific opinion. Guidance on the Post-Market Environmental monitoring (PMEM) of genetically modified plants The EFSA Journal, 9(8): 2316, 40 pp.	
Germany	BVL (German CA)	1. General comments	Comment 1 / Federal Office of Consumer Protection and Food Safety (German CA)	Application EFSA-GMO-RX-016 requests for renewal of the authorization of food and feed containing, consisting of, or produced from the genetically modified maize Bt11 and products other than food and feed containing or consisting of correspondent genetically modified plants for the same uses as any other maize with the exception of cultivation, authorized under Regulation (EC) No 1829/2003 (Commission Decision 2010/419/EU). The Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion	In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition,

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				that the data provided with application EFSA-GMO-RX-016 and the experience gained during the commercial use of maize Bt11 give no indication towards safety concerns and support the conclusion of the original risk assessment that maize Bt11 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses. Nevertheless, clarification with regard to the systematic search and evaluation of literature is recommended. Besides, the monitoring plan needs further elaboration.	the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).
Germany	BVL (German CA)	2.2. Post-market monitoring and post-market environmental monitoring reports	Comment 2 / Federal Office of Consumer Protection and Food Safety (German CA)	Case-specific-monitoring No adverse effects were identified according to the risk assessment and based on the monitoring reports provided by the applicant. Hence, a case-specific monitoring is currently not necessary. General surveillance The applicant provided yearly monitoring reports considering general surveillance from 2009 to 2017. The reports did not reveal any adverse effects (See also below: 4. Monitoring plan and proposal for improving the conditions of the original authorization).	The GMO Panel took note of the comments.
Germany	BVL (German CA)	2.3.1. Systematic search and evaluation	Comment 3 / Federal Office of Consumer Protection	According to the EFSA Guidance for renewal applications of genetically modified food and feed (EFSA, 2015) all scientific databases, relevant for the risk assessment in the field of molecular characterization, food and feed safety and the	The GMO Panel took note of the comment.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
		of literature	and Food Safety (German CA)	environment, should be searched for new scientific information in a comprehensive and structured manner. In the case under consideration, the applicant performed a literature search covering the database entry period from January 2008 to the date of the last reported database update prior to the search in 2018 taking into account the EFSA explanatory note on literature searching for GMO applications (EFSA, 2017). In this context, we draw attention to the following point: In the monitoring reports of the years 2009-2017, 92 literature references were identified to be relevant and consequently reported. Some of these references were also identified in the updated systematic literature search and their relevance was assessed in the context of the intended use. However, 70 literature references were not found in the updated systematic literature search. The applicant does not state the reason for this discrepancy. It is not comprehensible to what extent publications from the monitoring reports are covered by a possible specification of the search strategy in the current literature search. Therefore, the applicant should a) clearly explain and justify to what extent it was possible to cover and evaluate all relevant publications with the chosen strategy in the present case and b) explain the existing discrepancy between the different searches. If weaknesses are identified, the systematic search	

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				and evaluation of literature should be improved accordingly. EFSA (2015). Guidance for renewal applications of genetically modified food and feed authorised under regulation (EC) No 1829/2003 The EFSA Journal 13(6):4129 1-8. EFSA (2017). Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. EFSA supporting publications 2017:EN-1207. 48 pp. doi:10.2903/sp.efsa.2017.EN-1207	
Germany	BVL (German CA)	4. Monitoring plan and proposal for improving the conditions of the original authorisation	Comment 4 / Federal Office of Consumer Protection and Food Safety (German CA)	The monitoring plan is acceptable but needs further elaboration. Therefore, the applicant is recommended to revise the monitoring plan and present this revised monitoring plan together with a report one year after consent is given to be reassessed. 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	In the context of the assessment of several applications for the renewal authorisation of genetically modified (GM) plants for food and feed uses, import and processing, the environmental risk assessment (ERA) working group of the GMO Panel has been analysing the contents of the annual post market environmental monitoring (PMEM) reports as well as the relevance of their underlying monitoring methodology. The PMEM plans proposed by applicants consist of general surveillance of imported GM plant material. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on

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				the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate food and feed surveillance in coordination with the competent authorities. Information about the use of the product in food and feed could deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Therefore, the applicant should specify monitoring activities in the field of human and animal health. He should describe in detail how animal and human health surveillance is integrated in the monitoring plan. 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. 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.	the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).

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				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 would consider this information within General Surveillance. 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.	
Hungary	Ministry of Agriculture	1. General comments	HUN1	Bt11 GM maize expresses the transgenes a modified, truncated cry protein: Cry1Ab and the phosphinothricin acetyltransferase (PAT) protein for weed control. However, there are problems with the safety assessment of Bt11 maize: • The transgenes have no history of safe use The transgene Cr1Ab is a truncated, plant optimised cry protein. Neither the transgenic proteins in Bt11, nor their source organisms have a history of safe use, since they have never been consumed as food or feed, although they might have been in contact with humans and animals. • The transgenes might be glycosylated Transgenic cry proteins in plant are often glycosylated, increasing their allergic potential (Latham et al. (2017) The distinct properties of natural and GM cry insecticidal proteins, Biotechnology and Genetic Engineering Reviews, 33:1, 62-96) while the bacterial recombinant versions, used in all the tests, are not. The safety	The safety of Bt11 maize, including Cry1Ab and the PAT proteins was assessed in the frame of EFSA 2009. The GMO Panel acknowledges that no publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on maize Bt11 have been identified by the applicant. Reference: EFSA (European Food Safety Authority), 2009. Scientific Opinion on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta. EFSA Journal 2009;7(2):977, 13 pp. https://doi.org/10.2903/j.efsa.2009.977

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				testing should have been performed with the transgenic proteins from the GM maize. • There are receptors for cry proteins in mammals The applicant relies on the fact that cry proteins have no receptor on mammalian cells, contradicting scientific evidence on the existence of such receptors. Cry proteins are lectins, and even when they are present in a negligible amount, they are able to bind to cell surface receptors and exert biological/physiological/toxic/allergic effects, especially during long exposures. The mammalian gut and other cells contain Cry toxin receptors: the carbohydrates (GalNac and GlucNac), cadherins, and/or glycolipids, and other glycosylated membrane proteins acting as receptors (Vazquez-Padron et al. (2000) Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271, 54-58.; Ibrahim and Okasha (2016) Effect of genetically modified corn on the jejunal mucosa of adult male albino rats. Exp. Tox. Pathol. 68:579-588., Tayabali and Seligy (2000) Human cell exposure assays of Bacillus thuringiensis commercial insecticides: production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environ Health Perspect 108: 919-930.) The transgenic proteins do not degrade fully in the human or animal gut	

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				To prove the safety of Bt11 maize the applicant claims in the original Dossier that the transgenes are fully digested in the gut. In vitro digestibility studies (SIG and SIF) have no relevance to true digestibility in the bowel, especially for lectins. The data showing degradation of any transgenic proteins is no proof of their degradation in vivo. In vivo protein degradation in the gut can be measured. In cases when the protein is a lectin or an enzyme, after binding to its receptor(s)/substrate(s), the protein changes conformation to becomes resistant to proteolytic degradation. The cry proteins known to survive passage through the bowel, and degrade only partially to a functionally, biologically and immunologically active core proteins (Hilbeck and Otto (2015) Specificity and Combinatorial Effects of Bacillus Thuringiensis Cry Toxins in the Context of GMO Environmental Risk Assessment. Front. Environ. Sci. 3:71.; Latham et al. (2017) The distinct properties of natural and GM cry insecticidal proteins, Biotechnology and Genetic Engineering Reviews, 33:1, 62-96). In fact, transgenes originating from GM plants were detected in several animal organs (Nawaz et al. (2019) Addressing concerns over the fate of DNA derived from genetically modified food in the human body: A review. Food and Chemical Toxicology, 124: 423-430. https://doi.org/10.1016/j.fct.2018.12.030; Tudisco et al (2010) Fate of transgenic DNA and evaluation	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal 4: 1662-1671; Calabrò et al. (2015) Genetically modified soybean in a goat diet: Influence on kid performance. Small Ruminant Research 126: 67–74; Grønsberg et al. (2011). Uptake and organ distribution of feed introduced plasmid DNA in growing or pregnant rats. Food Nutrition Sci. 2, 377-386.), although the ability to detect them depend on the sensitivity of the methods used. Therefore, the degradation of the transgenic protein from the GM plant should have been determined experimentally. • Cry proteins are toxic Cry toxins (as their names tell us) are toxins, capable to binding to mammalian cells (Mezzomo et al. (2013) Hematotoxicity of *Bacillus thuringiensis* as Spore-crystal Strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss Albino Mice. J Hematol Thromb Dis 2013, 1:1) and exerting physiological/pathological effects and induce metabolic changes on their own (El-Shamei et al. (2012) Histopathological Changes in Some Organs of Male Rats Fed on Genetically Modified Corn (Ajeeb YG) Journal of American Science 2012: 8(10)); Tayabali and Seligy (2000) Human cell exposure assays of *Bacillus thuringiensis* commercial insecticides: production of *Bacillus cereus*-like cytolytic effects from outgrowth of spores. Environ Health Perspect 108 (10): 919-930.; Rubio-Infante and Moreno-Fierros (2016) An	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				overview of the safety and biological effects of *Bacillus thuringiensis* Cry toxins in mammals. Journal of Applied Toxicology, 36(5): 630-648.; Gab-Alla. et al. (2012) Morphological and biochemical changes in male rats fed on genetically modified corn (Ajeeb YG). J. Am. Sci. 8 (9): 1117–1123.), which clearly shows that the transgenic cry proteins expressed in plants are resistant to protein-degradation in vivo. The donor organism of this gene, *Bacillus thuringiensis* is toxic and allergic when humans are exposed to it. Cry toxins might enter the human body. Farm workers reported significantly greater health problems, ranging from fatigue to hair loss, and experienced significantly greater DNA and chromosomal damage, as assessed by a micronucleus frequency test of blood and buccal cheek samples, compared to a control group of non-agricultural workers (Venkata et al. (2016) Assessment of genotoxicity in female agricultural workers exposed to pesticides. Biomarkers 22 (5): 446-454.). Another paper evaluated data on the same persons and concluded that farm workers are at risks when exposed to cry proteins from transgenic crops (Flachs (2017) Transgenic cotton: High hopes and farming reality. Nature Plants 3). Cry toxins might be allergens and also adjuvants The allergenicity assessment ignores the fact that Bt toxins are allergens and adjuvants (Santos-Vigil et al. (2018) Study of the allergenic potential of *Bacillus thuringiensis* Cry1Ac toxin following intra-	

Country	Organization	Reference	Topic	Comment	GMO Panel responses
				gastric administration in a murine model of food-allergy. International immunopharmacology 61: 185-196. Vázquez-Padrón et al. (2000) Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from *Bacillus thuringiensis* HD 73 in mice. Brazilian Journal of Medical and Biological Research 33 (2): 147-155; Sagstad et al. (2007) Evaluation of stress- and immune-response biomarkers in Atlantic salmon, Salmo salar L., fed different levels of genetically modified maize (Bt maize), compared with its near-isogenic parental line and a commercial suprex maize. Journal of Fish Diseases 30 (4): 201–212.); El-Shamei et al. (2012) Histopathological Changes in Some Organs of Male Rats Fed on Genetically Modified Corn (Ajeeb YG) Journal of American Science 2012: 8(10). • The kernel and forage composition of Bt11 maize is different from its parent variety Several significant differences were found in composition of kernel and forage samples between Bt11 maize and its parent line (see original application).	
Hungary	Ministry of Agriculture	2.2. Post-market monitoring and post-market environmental	HUN2	The PMEM reports of Bt11 maize have never detected any problems either, similarly to other GM crops. It is stated in every single case that no unanticipated effects were observed. It is unfortunate that the applicant relies on the fact that cry proteins have no receptor in mammals, contradicting scientific evidence on the	The GMO Panel took note of this comment. The safety of Bt11 maize, including Cry1Ab and the PAT proteins was assessed in the frame of EFSA (2009). The GMO Panel acknowledges that no publications raising a safety concern for human and animal health and the environment which would change the original risk

Country	Organization	Reference	Topic	Comment	GMO Panel responses
		monitoring reports		existence of such receptors, see point 1; and ignores the fact that cry proteins known to survive passage through the bowel, and degrade only partially to a functionally, biologically and immunologically active core proteins, see point 1, to prove the safety of Bt11. Toxicity assessment ignores the facts that Cry proteins are lectins and even when they are present in a negligible amount are able to bind to cell surface receptors and exert biological/physiological/toxic/allergic effects, see point 1. The mammalian gut contains Cry toxin receptors: carbohydrates (GalNac and GlucNac) acting as receptors, cadherins, glycolipids, and/or other glycosylated membrane proteins, see point 1. The allergenicity assessment ignores the fact that Bt toxins are allergens and adjuvants, see point 1. Cry toxins might affect the consumer and non-target organisms as well lectins can also bioaccumulate and exert delayed toxic effects (Zdziarski et al. (2018) Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. Food and Nutrition Sciences 9: (6), 763-796.; Bøhn et al. (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140.).	assessment conclusions on maize Bt11 have been identified by the applicant. Reference: EFSA (European Food Safety Authority), 2009. Scientific Opinion on application reference EFSA-GMO-RX-Bt11 for renewal of the authorisation of existing products produced from insect-resistant genetically modified maize Bt11, under Regulation (EC) No 1829/2003 from Syngenta. EFSA Journal 2009;7(2):977, 13 pp.
Hungary	Ministry of Agriculture	2.3.2. Updated	HUN3	Update bioinformatics concluded that in silico amino acid translations of the sequences spanning the Bt11 insert DNA share no relevant amino acid	The applicant submitted updated bioinformatic analysis to identify ORFs with relevant similarity to toxins using up-to-date databases (spontaneous information 16/04/2020). The

Country	Organization	Reference	Topic	Comment	GMO Panel responses
		bioinformatics		sequence similarity to any known or putative mammalian toxins. However, Bt toxins are toxins, capable of binding to mammalian cells and should be listed in all toxin databases. It is very discouraging to find that these are missing from the updated toxin-, allergen- and antinutrient databases. It makes using bioinformatic analysis nearly hopeless to judge the safety of any of the transgenic protein. The applicant also stated that the Bt11 insert sequence was screened for identity to microbial DNA sequences no sequences able to promote homologous recombination between the Bt11 insert and any microorganisms were identified. Are not the Bacillus thuringiensis varieties listed in those databases?	applicant's toxin database was generated in-house by filtering the general UniProt database. It should be noted that Cry proteins were filtered out from the internal toxin database since self-identification of Cry proteins already known to be expressed by the insert would not add any value to the analysis.
				The calculation of potential risks for HGT occurring has been seriously underestimated. Calculating the chances of HGT ignores the fact that the transgenes in GM plants are usually plant optimized/synthetic versions of the genes occurring in Nature; they are expressed in a matrix different from that of their donor organism(s); the transgene(s) are under the influence of different regulatory elements aimed to maximize protein expression. Under these conditions HGT might occur with higher frequency, especially in the gut microbiome. Non-GM maize does not harm GM varieties, but GM maize has the ability to cross fertilize other non-GM maize varieties, therefore, GM plants	The applicant submitted updated bioinformatic HGT analysis using up-to-date databases (spontaneous information 16/04/2020). The analysis was performed according to EFSA guideline (EFSA, 2017). Given the results of this analysis and that the recombinant DNA in maize Bt11 does not confer selective advantages to microorganisms, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

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				might now be considered as invasive species (Paull (2018) Genetically Modified Organisms (GMOs) as Invasive Species. Geography and Spatial Sciences, Journal of Environment Protection and Sustainable Development. 4 (3): 31-37.)	
Hungary	Ministry of Agriculture	3. Overall assessment	HUN4	It is stated by the applicant that all the previous information submitted and remain valid and no amendments are necessary. However, Hungarian experts are still had not been satisfied that GM maize Bt11 is safe for the consumers and the environments. They consider the characteristics and the products derived from the Bt11 event to be different from those of conventional maize/parent variety. There is no consensus on the safety of GM crops (Krimsky (2015). An Illusory Consensus behind GMO Health Assessment. Science, Technology, & Human Values, pp. 1-32). In fact, there is indirect evidence to suggest that GM crops are not safe and have negative health effects (Smith (2017) Survey Reports Improved Health After Avoiding Genetically Modified Foods. Int J Hum Nutr Funct Med).	The GMO Panel takes note of the comment. In its scientific opinion on application EFSA-GMO-RX-016, the GMO Panel concluded that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize Bt11.
Netherlands	Dutch GMO Office	1. General comments	Dutch comment on EFSA/GMO/RX/016	The Dutch CA has assessed the renewal dossier with respect to the environmental, food and feed safety of Bt11 maize and has no comments or requests for additional information in relation to the safety of this GM event.	The GMO Panel thanks the Netherlands for the assessment.

Country	Organization	Reference	Topic	Comment	GMO Panel responses
Norway	VKM	3. Overall assessment	Norwegian Scientific Committee for Food and Environment (VKM)	VKM welcomes information on herbicide residue levels and their relevant metabolites in applications for herbicide tolerant GM-plants. Data on glufosinate-ammonium residue levels, including relevant metabolites, in plant material from the field studies would support the assessment of food, feed, and environmental safety.	The GMO Panel thanks Norway for the comment. It should be noted that the assessment of the herbicide residue levels is not in the remit of the GMO Panel.