Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_02	2.2.1 General description of the trait(s) and characteristics which have been introduced or modified Scientific Information, p. 23 The applicant confirms the presence of the antibiotic resistance gene nptII in maize MON87427xMON87460xMON89034x1507xMON87411x59122. This antibiotic resistance gene inactivates critically important aminoglycoside antibiotics (WHO 2012). Facing the current global crisis in antibiotic resistance (Neu 1992; French 2010; United Nations General Assembly 2016; WHO 2016; Martens and Demain 2017) it is irresponsible to fuel the environmental resistance gene pool with artificially and unnecessarily introduced variants of plant-derived nptII molecules and fragments thereof considering additionally the fact that this resistance gene could have been easily removed as it was placed in a cassette containing the cre/lox system. EFSA BIOHAZ Panel Members state in their Minority Opinion on the issue that "it would be imprudent to regard resistance to any antibiotic as being of little or no relevance to human health " and that for characterising the risk of the transfer of plant-derived antibiotic resistance marker genes as "high, low or unlikely, one needs to be able to estimate probabilities of antibiotic gene transfer from GM plants to bacteria. These probabilities are below the detection limits for the studies reported" (EFSA 2009). By placing this product on the market the applicant acts against the intention of Commission Implementing Regulation (EC) 503/13 which states in Recital 17 that "it is now possible to develop GMOs without the use of antibiotic resistance marker genes. Against this background and in accordance with Article 4(2) of Directive 2001/18/EC, the applicant should therefore aim to develop GMOs without the use of antibiotic resistance marker genes " (EC 2013). In the adult transgenic plant nptII has no function and is therefore to be characterised as superfluous DNA. Superfluous DNA should be avoided according to EFSA guidelines and the Commission Implementing Regulation (EC) 503/13: By placing this transgenic variety on the market the applicant is again violating Commission Implementing Regulation (EC) 503/13 which states that "the applicant shall endeavour to minimise the presence of inserted nucleic acid(s) sequences not essential to achieve the desired trait " (EC 2013). EFSA had noted a similar recommendation in its 2006 version of the guidance document for the risk assessment of genetically modified plants and derived food and feed (EFSA 2006).	The antibiotic resistance traits as present in GM plants and/or their derived products are evaluated on a case-by-case basis with respect to their safety for humans, animals and the environment by the GMO Panel (see EFSA, 2009b). The GMO panel has already assessed the presence of the *nptII* gene in MON 87460 and no safety concerns were identified (EFSA GMO Panel, 2012; 2019b). The GMO Panel took note of this comment. The applicant in accordance with Article 4(2) of Directive 2001/18/EC and with Reg (EU) 503/2013, should aim to develop GMOs without the use of antibiotic resistance marker genes. The EFSA GMO Panel assessed in previous opinions the probability and potential adverse effects of HGT of the recombinant DNA for the single events (see Table 2 in the Scientific Opinion) including the case of MON 87460 (EFSA GMO Panel, 2012; 2019b). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by HR. The GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this six-event stack maize to bacteria does not raise any environmental safety concern.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				[EC, 2013. Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48. EFSA, 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100. EFSA, 2009. Consolidated presentation of the joint scientific opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 1108: 1-8. French GL, 2010. The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 36 Suppl 3: S3-7. Martens E, Demain AL, 2017. The antibiotic resistance crisis, with a focus on the United States. J Antibiot (Tokyo). Neu HC, 1992. The crisis in antibiotic resistance. Science 257(5073): 1064-1073. United Nations General Assembly, 2016. Resolution adopted by the General Assembly on 5 October 2016: Political declaration of the high-level meeting of the General Assembly on antimicrobial resistance. http://www.who.int/antimicrobial-resistance/interagency-coordination-group/en/. WHO, 2012. Critically important antimicrobials for human medicine. 3rd revision 2011. WHO Press, World Health Organization, Geneva. http://www.who.int/foodborne_disease/resistance/cia/en/ (7 Oct 2013, date last accessed). WHO, 2016. Antimicrobial resistance: Fact sheet.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				http://www.who.int/mediacentre/factsheets/fs194/en/. Last accessed: April 5th, 2017]	
Austria	Fed.Ministry_Labour/Soc.A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_03	2.2.2 Information on the sequences actually inserted or deleted We would like to indicate that the applicant does not provide any data in the Scientific Information in support of the stability of the transgenic inserts in the stacked event, refers only to Reports by FROM CBI: [redacted] Study ID: 151065 and [redacted] Study ID: MSL0027161. In our opinion, this approach is very user unfriendly. It is apparent that the applicant uses at several occasions the expressions "similar", "are comparable" "should be conserved" when he refers to sequence comparison between single events and the corresponding sequence in the stack under evaluation in the Scientific Information. Taking a closer look for the referenced reports reveal the presence of mutations (for instance an SNP at position 5904 in the cry1F gene in the stack compared to the single event; see FROM CBI: [redacted] Study ID: 151065). These mutations are not indicated or discussed in the Scientific Information. We regard this as a substantial drawback in the presentation of the risk assessment by the applicant and as misleading. The applicant is requested to present such vital information in the Scientific Information and not try to hide it behind a bulk of raw data and company reports. This is especially annoying considering the fact that the applicant wastes a total of three pages describing standard procedures for conducting ELISAs properly (see Scientific Information, pages 27-29). We would like the EFSA GMO Panel to take note of this observation.	The GMO Panel takes note of the observation. Genetic stability is a requirement in the singles (GD/IR), while integrity is demonstrated in the stack. The applicant conducted the analyses based on the requirements for stacks and the GMO Panel was able to conclude on the integrity of the stacked events. The Scientific Opinion addresses all issues mentioned.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_04	2.2.3 Information on the expression of the inserted/modified sequence The notifier presents ELISA data for the concentrations of the six different Cry toxins (Cry1A.105, Cry1F, Cry2Ab2, Cry34Ab1, Cry35Ab1 und Cry3Bb1) as well as the PAT, CP4 EPSPS, NptII and CspB proteins in GM maize produced during field trials conducted at five locations in the US in 2014 (Scientific Information, p. 27). In addition, data are presented for the expression of DvSnf7-RNA. Results are presented for forage and grain derived from the GM maize stack and the concurrently grown single events - each treated with the respective complementary herbicide(s). However, the statistical analysis is restricted to basic descriptive statistics, such as means, data ranges, and standard deviation, an appropriate analysis of variance is lacking. However interactions between the various transgenes cannot be excluded. In our view this justifies a thorough assessment of the specific expression of transgenes in GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. This particularly applies to transgenes present in multiple copies, like the two CP4 EPSPS and the two PAT genes contained in this GM maize stack, but also to the combination of the various Cry proteins. E.g. the expression of the Cry1A.105 protein is obviously higher in the stacked GM maize than in the single event GM maize MON89034 (Scientific Information, Tab. 4) and a similar albeit smaller trend can be seen for some of the other Cry toxins in forage samples (Scientific Information, Tab. 5, 6, 8 & 9). In addition, the reference framework which is applied for the evaluation of potential differences in expression is not clear. According to the field trial design implemented by the notifier the reference is a comparison with expression of the respective transgenes in the single events. In this case EFSA's recommendations regarding equivalence testing should be applied, including the respective suggestions for the statistical analysis. However, this would require adaptations of the field trial design implemented by the notifier. According to a different approach a reference framework might be set by defining a maximum level of variation of expression for each single protein, which is considered acceptable. Also in this case additional data would be required, e.g. concerning the efficacy of the respective proteins. Therefore, we would appreciate a clarification concerning the	The EFSA GMO Panel acknowledges the comments. To clarify issues linked the newly expressed proteins the EFSA GMO Panel requested complementary information and clarifications which were used in the risk assessment and included in the scientific opinion. The applicant submitted the information in accordance with the explanatory note on the determination of newly expressed protein (EFSA 2018) and are in line with Commission Implementing Regulation (EU) No 503/2013. Interactions have been evaluated as indicated in the scientific opinion.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				reference framework to be used and recommend that EFSA requests a comparison of expression data based on a more detailed statistical analysis and based on the requirements in Implementing Regulation (EU) No 503/2013 (Annex II, 1.2.2.3.f) (EC 2013). We consider this of significant value for the exposure assessment and the toxicological assessment. Furthermore, we request that EFSA considers whether their recommendations regarding the statistical analysis of the comparative assessment, in particular the equivalence testing, would be applicable for the comparison of stacked event GMPs with single event GMPs. [EC, 2013. Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48.]	The EFSA GMO Panel acknowledges the comment and reminds that the proposed comparison is not required by the current guidance document.
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_05	2.2.4 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant Regarding the genetic stability of the inserts combined in GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 the notifier states that "There are no known mechanisms by which two inserts at different locations on different chromosomes could stimulate recombination on each other (if they do not express proteins involved in recombination pathways) " (Scientific information, p. 42). However, the data submitted by the notifier concerning the chromosomal locations of the various inserts (containing the different transgenes) in GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 are not sufficient to support this explanation. EFSA is requested to ask for additional information underlining the conclusion about genetic stability of inserts.	The EFSA GMO panel takes note of the comment. Integrity of events was demonstrated in the stack according to EFSA guidelines. The integrity and genetic stability of the inserts have been evaluated as described in the scientific opinion.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_06	2.2.5. Potential risk associated with horizontal gene transfer Scientific Information, p. 43 The applicant maintains that "it is highly unlikely, if not impossible, for DNA sequences from plants to recombine with genomic DNA in human or animal cells." We would like to indicate that there are several peer-reviewed reports available describing exactly this phenomenon (i.e. integration of food/feed/plant-derived DNA into the mammalian genome) (Schubbert et al. 1998; Deaville and Maddison 2005; Mazza et al. 2005). The applicant emphasises that "systemic barriers" (e.g. stomach acid, pancreatic nucleases, blood/systemic nucleases etc.) limit and/or eliminate the availability of exogenous DNA. We would like to indicate that there a many peer-reviewed publications available providing evidence that orally administered DNA is not completely degraded by gastrointestinal fluids for a certain period of time and survives - albeit reduced in length - the passage through the gastrointestinal tract (Schubbert et al. 1994; Schubbert et al. 1997; Schubbert et al. 1998; Martin-Orue et al. 2002; Netherwood et al. 2004; Wilcks et al. 2004; Sharma et al. 2006; Alexander et al. 2007) and that free extracellular DNA - in spite of blood nucleases - is present in the circulation and is used as valuable diagnostic marker (Anker and Stroun 2000). Plant DNA derived sequences especially from multi-copy (plastid) genes are detectable in blood and/or tissues after ingestion (Phipps et al. 2003; Deaville and Maddison 2005; Hanusová et al. 2007; Rehout et al. 2008; Bertheau et al. 2009; Spisák et al. 2013). Proteins and DNA are excellently protected against acidic conditions in the stomach and degradation by digestive enzymes if encapsulated by a plant cell wall (Kwon and Daniell 2016). The plant cell wall which is densely packed with lignin and cellulose provides natural protection against lysis because human enzymes are incapable to efficiently crack the glycosidic bonds of the plant cell wall carbohydrates (Cummings 1984). The content of a plant cell is therefore predominantly released only in the lower gastrointestinal tract where commensal bacteria provide the means to digest these plant cell walls (Sierk and Pearson 2004; Martens et al. 2011). This protective effect is exploited for the oral delivery of protein drugs which are "bioencapsulated" in plant cells and, thus, resistant to degradation in the upper gastrointestinal	The GMO Panel thanks Austria for this and the following comments on the potential risk associated with horizontal gene transfer. As reported in Section 6.1.1.2 of the EFSA GMO Panel Scientific opinion on MON87460 (EFSA GMO Panel, 2012) and Section 3.4.4.1 of the Scientific Opinion on MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (EFSA GMO Panel, 2019b), three different scenarios of integration of the transgenes of maize MON 87460 to bacteria in the environment were considered. 1. Mobilisation of *nptII* by the cre/lox system. The GMO Panel considered that the stabilisation of the *loxP-nptII-loxP* fragment due to the Cre recombination system present in bacteria containing a P1 or P1-like bacteriophage was unlikely. 2. Transfer of *nptII* by double homologous recombination to a Ti-plasmid of *A. tumefaciens*. In EFSA GMO Panel (2012) is also recognised that the acquisition of the *nptII* gene by bacteria without *nptII* genes could confer resistance to kanamycin or neomycin, and thus provide a selective advantage in habitats in which these antibiotics would be present. The updated bioinformatic analysis for MON 87460 did not result in new information which would change previous conclusions on possible HGT. It was confirmed the possibility for a facilitated double homologous recombination between the T-tr7 intervening sequence and the left border of the Ti cassette and the corresponding sequences in the *A. tumefaciens* Ti-plasmid downstream resulting in the insertion of the *nptII* expression cassette (*P35S/nptII/T-nos*). However, this led to the concomitant loss of a naturally occurring sequence in the *A. tumefaciens* Ti-plasmid resulting in a Ti-plasmid that would not promote for plant tumor formation (EFSA GMO Panel, 2012a). Due to the selective disadvantage of such bacterial recipients for growing in plants, and the natural abundance of *nptII* genes in the environmental bacterial communities, the GMO Panel concludes that there was no indication for a risk to human or animal health or to the environment. 3. Substitutive homologous recombination of *nptII* or *cspB* genes to the bacteria harbouring natural variants of such genes. The GMO Panel considered that if the *nptII* cassette from maize MON 87460 is transferred to bacterial cells, the expression of the gene cannot be excluded. In EFSA GMO Panel (2012a) is also stated that in case of

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				tract (Kwon and Daniell 2016). The applicant refers to a study performed by (Netherwood et al. 2004) and maintains that "a study conducted on human subjects fed on genetically modified maize has confirmed that the transgene does not survive passage through the human gastrointestinal tracts." This is not correct. The experiments have been performed with genetically modified soybeans. The obtained data are, thus, not representative for the present situation with maize which is to be risk assessed. Moreover, the applicant emphasises that "no evidence was found to suggest gene transfer between GM maize and intestinal micro-flora occurred during the feeding experiments" but forgets to mention that Netherwood et al. "showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel " (i.e. transfer of the transgenic epsps gene to bacteria) before the experiment (Netherwood et al. 2004). We would like to ask the EFSA GMO panel to take note of these observations. [Alexander TW, Reuter T, Aulrich K, Sharma R, Okine EK, Dixon WT, McAllister TA, 2007. A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production. Anim Feed Sci Technol 133(1-2): 31-62. Anker P, Stroun M, 2000. Circulating DNA in plasma or serum. Medicina (B Aires) 60(5 Pt 2): 699-702. Bertheau Y, Helbling JC, Fortabat MN, Makhzami S, Sotinel I, Audeon C, Nignol AC, Kobilinsky A, Petit L, Fach P, Brunschwig P, Duhem K, Martin P, 2009. Persistence of plant DNA sequences in the blood of dairy cows fed with genetically modified (Bt176) and conventional corn silage. J Agric Food Chem 57(2): 509-516. Cummings JH, 1984. Cellulose and the human gut. Gut 25(8): 805-810. Deaville ER, Maddison BC, 2005. Detection of transgenic and endogenous plant DNA fragments in the blood, tissues, and digesta of broilers. J Agric Food Chem 53(26): 10268-10275. Hanusová L, Vrabcová P, Rehout V, 2007. Detection of DNA fragments from feed containing GM organisms in blood of broilers. Genetics and Animal Breeding, Brno, Mendel	substitution of a natural *nptII* gene by the *nptII* gene of maize MON 87460 this would not confer a novel trait, and thus not provide an additional selective advantage. The updated bioinformatic analysis for MON 87460 (study REG-2020-0212 submitted to EFSA on the 29 May 2020) shows that there is no sufficient sequence identity and length of the codon-optimised cspB gene from B. subtilis with bacterial DNA for homologous recombination. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				University of Agriculture and Forestry Brno. Kwon K-C, Daniell H, 2016. Oral Delivery of Protein Drugs Bioencapsulated in Plant Cells. Mol Ther. Martens EC, Lowe EC, Chiang H, Pudlo NA, Wu M, McNulty NP, Abbott DW, Henrissat B, Gilbert HJ, Bolam DN, Gordon JI, 2011. Recognition and degradation of plant cell wall polysaccharides by two human gut symbionts. PLoS Biol 9(12): e1001221. Martin-Orue SM, O'Donnell AG, Arino J, Netherwood T, Gilbert HJ, Mathers JC, 2002. Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. Br J Nutr 87(6): 533-542. Mazza R, Soave M, Morlacchini M, Piva G, Marocco A, 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Res 14(5): 775-784. Netherwood T, Martin-Orue SM, O'Donnell AG, Gockling S, Graham J, Mathers JC, Gilbert HJ, 2004. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat Biotechnol 22(2): 204-209. Phipps RH, Deaville ER, Maddison BC, 2003. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci 86(12): 4070-4078. Rehout V, Hanusová L, Čítek J, Kadlec J, Hosnedlová B, 2008. Detection of DNA fragments from Roundup Ready soya in blood of broilers. Journal of Agrobiology 25: 145-148. Schubbert R, Hohlweg U, Renz D, Doerfler W, 1998. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet 259(6): 569-576. Schubbert R, Lettmann C, Doerfler W, 1994. Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Mol Gen Genet 242(5): 495-504.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Schubbert R, Renz D, Schmitz B, Doerfler W, 1997. Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. PNAS 94(3): 961-966. Sharma R, Damgaard D, Alexander TW, Dugan MER, Aalhus JL, Stanford K, McAllister TA, 2006. Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem 54(5): 1699-1709. Sierk ML, Pearson WR, 2004. Sensitivity and selectivity in protein structure comparison. Protein Sci 13(3): 773-785. Spisák S, Solymosi N, Ittzés P, Bodor A, Kondor D, Vattay G, Barták BK, Sipos F, Galamb O, Tulassay Z, Szállási Z, Rasmussen S, Sicheritz-Ponten T, Brunak S, Molnár B, Csabai I, 2013. Complete Genes May Pass from Food to Human Blood. PLoS One 8(7): e69805. Wilcks A, van Hoek AH, Joosten RG, Jacobsen BB, Aarts HJ, 2004. Persistence of DNA studied in different ex vivo and in vivo rat models simulating the human gut situation. Food Chem Toxicol 42(3): 493-502.]	
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.2 Information relating to the genetically modified plant	AUT Comment_07	2.2.5. Potential risk associated with horizontal gene transfer Scientific Information, p. 44 The applicant maintains that "there are no reports plant genomic DNA integrating into the genome of a consuming human or animal." This is not correct. Plant-derived DNA sequences especially from multi-copy (e.g. plastid) genes are detectable in blood and/or tissues after ingestion (Phipps et al. 2003; Deaville and Maddison 2005; Hanusová et al. 2007; Rehout et al. 2008; Bertheau et al. 2009; Spisák et al. 2013) and Schubbert et al. are reporting of orally ingested foreign DNA which was subsequently found associated with mammalian chromosomal DNA (Schubbert et al. 1998). The applicant describes a scenario of factors which in his opinion have to occur concomitantly before horizontal gene transfer from genetically enhanced plants to environmental micro-organisms gains any significance and, thus, insinuates that all these factors are highly unlikely to occur in natural environments. We refute this line of argumentation by	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				discussing the relevance of each mentioned factor for HGT (please see below): 1) "The recipient bacteria must be competent and able to accept exogenous DNA." The applicant seems to imply that there are probably no competent bacteria available in environments exposed to plant-derived transgenic inserts of microbial origin. We would like to indicate that this is no question of whether competent bacteria are present at all but when and under which circumstances bacteria become competent for DNA uptake. Competence is conserved in at least six different phyla and an old pathway in evolutionary terms (Lorenz and Wackernagel 1994; Johnsborg et al. 2007; Zaccaria et al. 2014). Many bacterial genera and families are carriers for competence genes. For more than 80 bacterial species experimentally proven data for their transformability in natural environments are available (Johnston et al. 2014). A more recent survey collected experimental data for natural transformability of more than 130 bacterial species (Woegerbauer et al. 2015). It was demonstrated that for instance probably all members of the gamma-proteobacterial section of the domain bacteria contain the signature of genes involved in the development of competence and uptake of free extracellular DNA (Cameron and Redfield 2006). The genes for the DNA uptake machinery are therefore putatively present throughout the bacterial and archaebacterial domains of the tree of life (Claverys and Martin 2003; Woegerbauer et al. 2015). It is only a matter of time to establish the conditions which induce the activation of these respective competence genes in natural environments (conditions which may vary even from species to species (Seitz and Blokesch 2013; Johnston et al. 2014)). 2) "The recipient bacteria and donor plant must share DNA that is homologous." We would like to indicate that most transgenic inserts mediating the desired phenotype in the stacked event under present evaluation are of bacterial origin and are per definitionem homologous to their counterparts present in naturally occurring plant-associated, soil and gut bacteria and, thus, do "share DNA that is homologous". The applicant perpetuates a definition for homologous sequences focusing on a threshold of "at least two 70 bp of DNA sequences having at least 67 identical nucleotides" between incoming and receiving (genomic) DNA. We would like to indicate that these numbers are arbitrarily chosen and it is questionable if these set boundaries are of any biological	1,2,3) Genomic DNA can be a component of food/feed products derived from maize. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and domesticated animals, and in other environments may be exposed to fragments of DNA, including the recombinant fraction of such DNA. Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally-located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009). The only mechanism known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes is homologous recombination. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties. The EFSA GMO Panel assessed in previous opinions the probability and potential adverse effects of HGT of the recombinant DNA for the single events (see Table 2 in the Scientific Opinion). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by HR.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				relevance. Analyses of bacterial sequence databases using these set of parameters for the query are most likely completely irrelevant concerning the potential of the transgenic inserts to be transferred to bacterial receptor strains in natural environments. In this respect we would like to mention that EFSA is also recommending a different – unfortunately also suboptimal – approach to check the potential of transgenic insert sequences for their potential to undergo homologous recombination with genomic sequences endogenously present in exposed bacterial populations (EFSA 2015). 3) "The sequence between the two homologous regions in the bacterial genome cannot contain essential genes that if lost due to recombination would be lethal or otherwise compromise the fitness of the recipient bacteria." The applicant appears to be fixed on a model of recombination which relies on a substitutive replacement of genomic sequences as the only possible result of the process. We are of the opinion that the applicant is describing the situation in a much to narrow fashion. It appears that he does not take into account the possibility of homology-directed/facilitated illegitimate recombination (HFIR) as mechanism which may support the dissemination of prokaryotic genes and gene fragments in bacterial populations. Although HFIR indeed may be an extremely rare event under naturally occurring conditions it nevertheless may be of decisive significance for the risk assessment of HGT in bacterial populations under strong selection pressure (Heinemann and Traavik 2004). Glyphosate is interfering with bacterial growth and is acting as antimicrobial agent under certain circumstances leading to shifts in bacterial community structures (Araujo et al. 2003; Shehata et al. 2013; Arango et al. 2014; Kurenbach et al. 2015). Glyphosate may therefore act as potent selector for the acquisition of plant-derived transgenic epsps homologs. The most outstanding feature of HFIR is that it is relying only on a single anchor sequence which should provide a homologous region of approx. 150 bp (Acinetobacter baylyi) or 180 bp (Streptococcus pneumoniae) with 100% sequence identity to the recombination target sequence and a short region of microhomology (3-10 bp) with relaxed requirements for sequence complementarity on the opposite end of the incoming DNA strand (de Vries and Wackernagel 2002; Prudhomme et al. 2002; de Vries and Wackernagel 2004). Both requirements are much less stringent compared to the	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				thresholds as defined by the relevant scientific note delivered by EFSA on this topic (i.e. significant alignments should meet a threshold of 95% identity in alignments of at least 200 bp in length and have at least two regions of similarity between the incoming DNA fragment and the receiving genomic or extrachromosomal microbial sequence (EFSA 2015). These limits reduce the sensitivity of the sequence alignment search for biologically relevant recombination partner molecules significantly. EFSA indicates that HFIR has not been observed under field conditions. However, the currently available tools for monitoring horizontal gene transfers in natural environments are inadequate to capture rare events (i.e. the sensitivity of the available methodology is too low) (Nielsen and Townsend 2004; Townsend et al. 2012; Nielsen et al. 2014). Assuming an already extremely rare recombination/HGT plant to bacterial DNA transmission frequency of 10E-15 under naturally occurring conditions each square meter of an ordinary agricultural field would harbour at least one recombinant cell. This would accumulate to a total number of 10E12 recombinants/field. Nevertheless, 3 tons of soil would have to be analysed to detect one recombinant cell with the available technology (Heinemann and Traavik 2004). Both numbers (transmission frequency, amount of soil to be tested) currently exceed any detection limit and laboratory capacity by several orders of magnitude (Nielsen et al. 2014). Additionally, it must be stressed that frequency estimates for horizontal gene transfer are not predictive for long-term effects (Pettersen et al. 2005). In summary, we would like to indicate that complete replacement of an endogenous gene or an essential part of it (and thereby destroying its function) by recombination is not the only possible outcome of homologous recombination. Gene transfer and exchange processes relying on HFIR provide a means for genetic variability allowing bacteria to easily adapt to changing environmental conditions (de Vries and Wackernagel 2002; Prudhomme et al. 2002; Woegerbauer et al. 2015). 4) "Assuming recombination has occurred, the gene transferred from the plant genome must provide an advantage to the recipient bacteria in the environment over its untransformed neighbors." The applicant is insinuating that there would be no selection pressure in natural environments and the transgenic inserts of prokaryotic origin would not provide any selective advantage if take up by a bacterial recipient. In the case of epsps quite the opposite is true on	4) The GMO Panel took note of this comment and reminds that the scope of this application is for import/processing for food/feed uses, excluding cultivation.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				agricultural fields where soil and plant-associated bacterial populations are under strong selection pressure by glyphosate. This may facilitate the selection, survival and establishment of rare gene transfer events in exposed bacterial populations which may then be affected by shifts in their community structure (Busse et al. 2001; Araujo et al. 2003; Kremer et al. 2005; Kuklinsky-Sobral et al. 2005; Ratcliff et al. 2006; Kremer and Means 2009; Barriuso et al. 2010; Lancaster et al. 2010; Barriuso et al. 2011a; Barriuso et al. 2011b; Zobiole et al. 2011; Lane et al. 2012; Krüger et al. 2013; Shehata et al. 2013; Arango et al. 2014; Karki and Ham 2014; Allegrini et al. 2015; Kurenbach et al. 2015). The applicant is referring to "FASTA searches of databases containing bacterial and archaea genomes, naturally occurring plasmids and viral (including bacteriophage sequence) DNA sequences." We would like to indicate that according to Report MSL0027378 (FROM CBI: ([redacted])) only 4905 bacterial genomes were available in the respective database which was used for the analysis of potential recombination partner molecules. Considering the fact that it was estimated that 1 g of soil may contain 10,000 (Torsvik et al. 2002) to more than 10 million of 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. The relevance of this bioinformatic approach for assessing the risk of horizontal gene transfers via transformation is therefore highly questionable. The applicant concludes that "it is highly unlikely, if not impossible, for DNA sequences from plants to recombine with genomic DNA in cells of […] microorganisms." We would like to indicate that this conclusion is most likely correct considering endogenous plant DNA sequences, but it is most likely irrelevant considering transgenic inserts of microbial origin, because these prokaryotic sequences of constitute optimal recombination partners with bacterial chromosomes. We would like to ask the EFSA GMO Panel to take note of these observations. [Allegrini M, Zabaloy MC, Gomez ED, 2015. Ecotoxicological assessment of soil microbial community tolerance to glyphosate. Sci Total Environ 533: 60-68. Arango L, Buddrus-Schiemann K, Opelt K, Lueders T, Haesler	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
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Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
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Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
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Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				transfer events in bacterial populations. Front Microbiol 4: 415. Nielsen KM, Townsend JP, 2004. Monitoring and modeling horizontal gene transfer. Nat Biotechnol 22(9): 1110-1114. Pettersen A-K, Bøhn 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. Phipps RH, Deaville ER, Maddison BC, 2003. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci 86(12): 4070-4078. 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. Ratcliff AW, Busse MD, Shestak CJ, 2006. Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Applied Soil Ecology 34(2–3): 114-124. Rehout V, Hanusová L, Čítek J, Kadlec J, Hosnedlová B, 2008. Detection of DNA fragments from Roundup Ready soya in blood of broilers. Journal of Agrobiology 25: 145-148. Schubbert R, Hohlweg U, Renz D, Doerfler W, 1998. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet 259(6): 569-576. Seitz P, Blokesch M, 2013. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. Shehata A, Schrödl W, Aldin AA, Hafez H, Krüger M, 2013. The Effect of Glyphosate on Potential Pathogens and Beneficial Members of Poultry Microbiota In Vitro. Curr Microbiol 66(4): 350-358. Spisák S, Solymosi N, Ittzés P, Bodor A, Kondor D, Vattay G, Barták BK, Sipos F, Galamb O, Tulassay Z, Szállási Z,	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Rasmussen S, Sicheritz-Ponten T, Brunak S, Molnár B, Csabai I, 2013. Complete Genes May Pass from Food to Human Blood. PLoS One 8(7): e69805. Torsvik V, Ovreas L, Thingstad TF, 2002. Prokaryotic Diversity--Magnitude, Dynamics, and Controlling Factors. Science 296(5570): 1064-1066. Townsend JP, Bohn T, Nielsen KM, 2012. Assessing the probability of detection of horizontal gene transfer events in bacterial populations. Front Microbiol 3: 27. Woegerbauer M, Kuffner M, Kopacka I, Domingues S, Steinwider J, Nielsen KM, Fuchs K, 2015. Impact of mosaic genes on the risk assessment of GMOs. Federal Ministry of Health: 1-268. Zaccaria E, van Baarlen P, de Greeff A, Morrison DA, Smith H, Wells JM, 2014. Control of competence for DNA transformation in Streptococcus suis by genetically transferable pherotypes. PLoS One 9(6): e99394. Zobiole LHS, Kremer RJ, Oliveira RS, Constantin J, 2011. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. J Appl Microbiol 110(1): 118-127.]	
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects	AUT Comment_08	2.3.2. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms Scientific Information, p. 45 In this section the applicant neglects any relevance of the transgenic inserts of microbial origin for horizontal plant to bacteria gene transfer and explains his no-risk-hypothesis by the absence of genetic elements with "a genetic transfer function". The inserted gene cassettes may indeed lack conventional genetic elements coding for proteins typically involved actively in horizontal gene transfer processes (like tra or vir operons). However, this section is clearly headed by the title "Any change to the ability of the genetically modified plant to transfer genetic material to other organisms." Concerning plant to bacteria gene transfer natural genetic transformation is a core mechanism for horizontal gene transfer (Stewart 1992; Lorenz and Wackernagel 1994; Chen and Dubnau 2004; Johnsborg et al. 2007). Bacterial transformation in general is	The GMO Panel took note of these observations.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				relying on the presence of 2 crucial elements: 1) Free extracellular (donor-) DNA, and 2) Competent bacterial (receptor-) cells (Dubnau 1999; Chen et al. 2005; Thomas and Nielsen 2005). The presence of genes coding for "genetic transfer functions" on the donor-DNA strand is absolutely no requirement for successfully transforming bacteria (and, thus, spreading genetic information from the transgenic plant to other organisms). In contrast to the initial statement of the applicant quite the opposite is true: Even the mere presence of bacterial sequence context in the transformed plant genome increases significantly the ability of the genetically modified plant to exchange the respective information with bacterial recipients compared to its non-modified conventional counterpart. We would like to ask the EFSA GMO Panel to take note of these observations. [Chen I, Christie PJ, Dubnau D, 2005. The ins and outs of DNA transfer in bacteria. Science 310(5753): 1456-1460. Chen I, Dubnau D, 2004. DNA uptake during bacterial transformation. Nature Reviews Microbiology 2(3): 241-249. Dubnau D, 1999. DNA uptake in bacteria. Annual Rev Microbiol 53: 217-244. Johnsborg O, Eldholm V, Havarstein LS, 2007. Natural genetic transformation: prevalence, mechanisms and function. Res Microbiol 158(10): 767-778. Lorenz MG, Wackernagel W, 1994. Bacterial gene transfer by natural transformation in the environment. Microbiol Mol Biol Rev 58: 5563-5602. Stewart GJ, 1992. Gene transfer in the environment: transformation. Release of genetically engineered and other micro-organisms. Fry, J. C., Day, M. J., Martin, M. J. Cambridge University Press, Cambridge: 82–93. Thomas CM, Nielsen KM, 2005. Mechanisms of, and barriers to, horizontal gene transfer between bacteria. Nature Reviews Microbiology 3(9): 711-721.]	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	AUT Comment_09	The data presented for the comparative analysis were generated in field trials conducted in North America at eight trial sites in 2014. The trial included untreated plots and plots treated with the complementary herbicides (one application of glyphosate followed by one application of glufosinate). However, no detailed information regarding the selection of the trial sites is presented and no other data than basic data on climatic conditions, soil type and use of maintenance chemicals are provided to characterise the test sites (FROM CBI: Study MSL0027664 [redacted]). The submitted data are considered insufficient to establish that the trial sites are representative as regards agronomic practices or abiotic (e.g. soil moisture, soil fertility) and biotic factors (e.g. prevailing pest and disease pressure, weed profiles). According to available guidance by EFSA (EFSA 2010; EFSA 2015) and Implementing Regulation (EU) No 503/2013 (EC 2013) a justification shall be provided to demonstrate that the trial sites and conditions are representative of the range of receiving environments, where the crop will be commercially grown, explicitly justifying the choice of sites (EFSA 2010). Thus, we request that the notifier provides further information concerning the selection of sites. [EC, 2013. Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48. EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111. EFSA, 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. The EFSA Journal 13(6):4128: 1-44.]	The field trials were conducted in typical maize growing areas of the USA, representing regions of diverse agronomic practices and environmental conditions, which is supported by the geographic map indicating the locations, the information provided on the variety of agronomic practice, soils and meteorological factors. In order to improve the representativeness of the selected field trials, EFSA published a guidance document on the agronomic and phenotypic characterisation of genetically modified plants (EFSA GMO Panel, 2015a). Application EFSA-GMO-DE-2017-139 was submitted during the transitional period of the GMO Panel guidance. Therefore, the requirements of the guidance document were not fully applicable for this application. Additional information to further describe the selection of sites and the agronomic management practices applied were provided on 3/9/18. The GMO Panel concludes that the geographical locations, soil characteristics, meteorological conditions and management practices of the field trials are typical for receiving environments where the test materials could be grown.
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.3.4 Comparative analysis of composition	AUT Comment_10	The scope of the comparative analysis concerning food and feed risk assessment conducted by the notifier for GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 is considered too narrow in several respects. The cultivation of MON87427xMON87460xMON89034x1507xMON87411x59122	The GMO Panel thanks Austria for this comment and takes note of these observations, however the assessment of herbicide residues and metabolites is not in the remit of the GMO Panel. In relation to the use of intended herbicides on the treated plots of

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				enables the application of different types of complementary herbicides based on glufosinate and glyphosate as active ingredients. Both the PAT as well as the CP4 EPSPS protein are expressed from two copies of transgenes combined in this GM maize stack. This might allow for the application of higher amounts of glufosinate and glyphosate based herbicides contributing to higher levels of herbicide residues and metabolites in harvested crop material, which constitutes an issue of concern (Cuhra 2015; Myers et al. 2016). Furthermore, it is unclear whether the application rates used in the field trials correspond to common usage of glyphosate and glufosinate based herbicides in the US and other countries where this GM maize stack is cultivated for future export, e.g. to the EU. The marketing of glufosinate in the EU will cease in 2019 and the MRLs for products cultivated in the EU subsequently will be lowered to the limit of quantification (LOQ) (pers. communication AGES). Further data is needed in order to assess whether accumulation of glufosinate and glyphosate residues and metabolites may occur in commercially produced GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 and whether unacceptable levels of such residues and metabolites may be contained in the respective GM products. Currently, MRLs of 1 mg/kg for glyphosate and of 0.1 mg/kg for glufosinate are established in maize imported from third countries (EC 2013; EC 2016). Therefore, the notifier should be requested to demonstrate that these MRLs are not exceeded in maize grain from GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. In addition, we note that any differences and non-equivalences identified in the compositional analysis (e.g. for manganese, glutamic acid, proline) were only identified in GM material treated with the complementary herbicides (Scientific Information, Tab. 13). Therefore, we recommend EFSA to request a broader data basis with respect to the compositional analysis including an analysis of residual herbicides and metabolites in order to draw sound conclusions concerning potential differences of food and feed products derived from GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 with products derived from conventional maize. A transcriptomics analysis of unintended effects of the expression of DvSnf7-RNA could provide indications whether	maize MON 87427 x MON 87460 x MON 89034 x 1507 x MON 87411 x 59122, the EFSA GMO Panel verified that the application rate was within the range recommended by the manufacturers. In addition, the EFSA GMO Panel reminds that the herbicide management strategy adopted on the GM maize exposed to the intended herbicides consists in GM plants treated with the intended herbicides in addition to the full conventional herbicide regime included in the field management (additional treatment). The EFSA GMO Panel assessed the possible unintended effects of MON 87411 and the expression of DvSnf7 dsRNA in the frame of application EFSA-GMO-2015-124 (EFSA GMO Panel, 2018a) where the in planta RNAi off-target search, performed with the sequence of the

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				other metabolic functions are affected and whether such effects may result in compositional changes of constituents which are not assessed during the comparative assessment (Heinemann et al. 2013). The notifier assumes that no potential off-target effects would result from the expression of DvSnf7 dsRNA in GM maize MON87427xMON89034xMIR162xMON87411 (Scientific Information, p. 42; FROM CBI: [redacted] Study RAR-2016-0119), however this assumption should be confirmed by an assessment as recommended above. [Cuhra M, 2015. Review of GMO safety assessment studies: glyphosate residues in Roundup Ready crops is an ignored issue. Environmental Sciences Europe 27: 20. EC, 2013. Commission Regulation (EU) No 293/2013 of 20 March 2013 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for emamectin benzoate, etofenprox, etoxazole, flutriafol, glyphosate, phosmet, pyraclostrobin, spinosad and spirotetramat in or on certain products. Official Journal of the European Union. L 96: 1-30. EC, 2016. Commission Regulation (EU) 2016/1002 of 17 June 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for AMTT, diquat, dodine, glufosinate and tritosulfuron in or on certain products. Official Journal of the European Union. L 167: 1-45. Heinemann JA, Agapito-Tenfen SZ, Carman JA, 2013. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environment International 55: 43-55. Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, Hansen M, Landrigan PJ, Lanphear BP, Mesnage R, Vandenberg LN, Vom Saal FS, Welshons WV, Benbrook CM, 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environ Health 15(1): 19.]	DvSnf7 dsRNA, did not provide indication for an off-target effect that would require further safety assessment. This conclusion has been confirmed by the updated bioinformatic analysis submitted on 29/05/2020 and 16/7/2020. In addition, the agronomic, phenotypic and comparative analysis of maize MON 87427 x MON 87460 x MON 89034 x 1507 x MON 87411 x 59122 does not provide indication of unintended effects.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.3.4 Comparative analysis of composition	AUT Comment_11	Statistical significant differences In the year 2014, GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 was planted in US field trials for compositional analysis. The field trials were conducted at eight sites and included the GM maize stack, its non-GM near-isogenic comparator (control line MPA640B), and a total of 18 reference maize varieties. Two different treatments were included in the trial design and statistical analysis: • GM stacked maize treated with intended herbicides - glyphosate and glufosinate (T) • GM stacked maize treated with conventional herbicides but not treated with glyphosate and glufosinate (NT) A total number of 78 analytes in maize grain and forage was tested in each of the two treatment groups (T, NT) in compositional analysis. 15 analytes had 50% or more sample values below the LLOQ and were not categorised. 63 analytes were categorised in each of the treatment types (T, NT) (FROM CBI: [redacted] Study MSL0026704, p. 12). For the GM stack treated (T) 40 of these 63 analytes were statistically significantly different (at a 90% confidence level) as compared to the control line (= 63% of the analytes). For the GM stack not treated (NT) 25 of these 63 analytes were statistically significantly different (at a 90% confidence level) as compared to the control line (= 42% of the analytes). On average half of the analytes were significantly different at the across-site analysis. The treatment of the GM stack with the intended herbicides glyphosate and glufosinate seems to have influenced the statistical results by increasing the number of differences between the GM maize and its control line. Nevertheless, even the GM stack (NT) has significant differences in more than 40% of the analytes. This is more than would be expected from the statistical model (at a 90% confidence level). The result also shows a number of trends in the significant differences: • Protein and several amino acids are significantly higher in the GM maize than in the control line in both treatment groups. The concerned amino acids are alanine, aspartic acid, glutamic acid (type 6), isoleucine (type 6), leucine (type 6), and proline (type 7). • A number of mineral are significantly different in the GM maize in both treatment groups: manganese is significantly	The GMO Panel assessed all significant differences between the six-event stack maize and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM maize reference varieties. None of the differences identified in forage and grain composition between the six-event stack maize and the non-GM comparator needed further food/feed safety assessment except for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. The relevance of these changes was further discussed in Section 3.4.3.

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				higher (rel. difference 12.8%), calcium significantly lower (rel. diff. 9.1%, 10.9%), copper is significantly lower (rel. diff. 15.3%, 11.8%), and iron is also significantly higher (rel. diff. 8.0%, 4.7%). • A couple of vitamins shows high relative differences with trends in significant differences: vitamin A is significantly lower in the GM maize (rel. diff. 22.4%, 23.0%), and vitamin E is also significantly lower (rel. diff. 8.2%, 9.8%). It is acknowledged that several analytes showing significant results are discussed by comparing both absolute differences and mean values. However, in most cases the notifier explains significant differences being of no relevance because of overlapping ranges of the GM maize stack and the control line (conventional counterpart). However, high natural variation in analytes can lead to overlapping ranges of maize plant lines that are substantially different. It would be more consistent to discuss the results for each analyte based on relative differences of means (and not the absolute differences) between the GM maize and the control lines. We also would like to point out that "outcome types 1 or 2 may easily be obtained for characteristics that are stable and precisely measured within each genotype, but that have a large natural variation among commercial genotypes " (EFSA 2010). The notifier should, therefore, provide an analysis of the observed natural variation of the type 2 analytes that are significantly different. The notifier misses to discuss each statistically different analyte, and thereby misses to provide answers on metabolic shifts resulting from genetic modification of GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. Although significant differences or trends do not mean a risk per se, a large number of significances indicates that genetic modification has resulted in unintended effects. Such effects might have the potential to be harmful. The percentage of significances in the compositional analysis is relatively high: In GM maize (T) and GM maize (NT), 63% and 43%, respectively. It should be taken into consideration that health risks associated with changed pattern of minor metabolites (e.g. plant hormones) may not be verifiable during compositional analysis, because evaluation is limited to the standard compounds outlined in OECD consensus documents. Moreover, the comparative assessment, in general, could be	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				strengthened by direct comparison (in form of a table or chart) and discussion of results for the GM maize stack and the six GM maize single events. [EFSA, 2010. Scientific opinion of the GMO Panel on statistical considerations for the safety evaluation of GMOs. The EFSA Journal 8(1):1250: 1-59.]	
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	AUT Comment_12	Field trials were conducted at eight locations in the United States for one-season. The trial design complies with EU standards. 13 agronomic characteristics were evaluated in the assessment, which generally can be considered sufficient. The measured data of all locations seem to be plausible. However, it is notable that the endpoints "Abiotic Stressor", "Disease Damage" and "Arthropod Damage Evaluation" show hardly any differences between the GM maize stack and the control line. And the presentation of the results (FROM CBI: Study MSL0027664. 2016a, p. 45-47) is highly intransparent as it lacks details such as single location results.	The GMO Panel, in light of the scope of the application, considered that the information provided was adequate.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.4 Toxicology	AUT Comment_13	The assessment of toxic effects is based on the risk assessments conducted previously for the single events used to generate GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 and the comparative assessment of the stacked GM maize. In our view these assessments show the following shortcomings: • With the exception of the obligatory 90-day food safety studies the assessment of toxic effects in the respective single events is based on studies, which were conducted with microbially-produced Cry-toxins (see our comments to the respective single event applications). However relevant differences between Cry-toxins expressed in GM maize products (including modified or chimeric Cry-proteins), microbially-produced Cry proteins used in toxicological tests and naturally occurring Cry-proteins exist concerning various properties (for discussion of respective differences see (Latham et al. 2017)). • Neither whole plant toxicity studies with GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 are presented nor specific studies to test for potential combinatory effects of all transgenes contained in this GM maize stack. Nevertheless the notifier claims that the newly expressed proteins 'have a history of safe consumption' and 'have no synergistic or antagonistic effects to each other' (Scientific Information, p. 59). • Herbicide residues and metabolites contained in GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 are neither considered in the assessment of toxic effects nor in the compositional assessment used by the notifier to argue that a 90-day feeding study in rodents is not necessary (Scientific Information, p. 60). However, for example data established for glyphosate resistant soybeans containing residues of glyphosate and its main metabolite AMPA showed significantly negative effects on a range of life-history traits of Daphnia magna (Cuhra et al. 2015). Additionally, reviews address potential risks associated with the accumulation of herbicide residues in the food chain and the environment (Bai and Ogbourne 2016) and suggest that low concentrations (i.e. below regulatory limits) of glyphosate and glyphosate based herbicides may result in risks to human health (Mesnage et al. 2015). A recent long-term study in rats found substantial adverse hepatic effects after chronic exposure to an ultra-low dose of glyphosate (Mesnage et al. 2017). EFSA recommends that the risk assessment of stacked event	The GMO Panel thanks Austria for the comments. The assessment of stacked event is in line with Regulation (EU) No 503/2013. It is based on the previous assessment of the single events composing the stacked GMP and on the evaluation of interactions between the events. • Consistently, the assessment of the protein newly expressed in a stack is based on the assessment of these in the context of the respective single event plants, updated bioinformatics, additional information, if any, and on considerations on their possible interactions of relevance for food and feed safety. In general, the use of recombinant "surrogate" proteins for safety studies is in line with Reg (EU) 503/2013. The equivalence of the "surrogate" protein to the plant expressed protein is thoroughly evaluated, and differences, if any, are discussed with regards to their possible impact on the adequacy and representativeness of the test material used in safety studies. The GMO Panel agrees with Austria on the technical challenges offered in the identification of recombinant Cry proteins equivalent to the plant ones; these have been taken into account in the context of the assessment of the respective single-event maize. • The assessment of potential interactions of relevance for food and feed safety has been conducted, and it is based on the biological function of the proteins newly expressed in the six-event stacked maize (see section 3.4.3.3 of the Scientific Opinion for further details). Based on this, no further studies were deemed necessary by the GMO Panel. • The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				GMPs should focus on potential interactions between the traits (EFSA 2010). Therefore, studies to test mixtures of the transgenic proteins expressed in stacked events should be conducted and in planta tests with the stacked event in tier-1 studies should be included (EFSA 2010). These recommendations by EFSA should be implemented for a comprehensive assessment since no other agreed test approach for combinatorial effects is available and the level of herbicides residues and metabolites was not addressed during the compositional assessment (see comment to 1.3.4). [Bai SH, Ogbourne SM, 2016. Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. Environ Sci Pollut Res Int 23(19): 18988-19001. Cuhra M, Traavik T, Dando M, Primicerio R, Holderbaum DF, Bøhn T, 2015. Glyphosate-residues in Roundup-Ready soybean impair Daphnia magna life-cycle. Journal of Agricultural Chemistry and Environment 4: 24-36. EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111. Latham JR, Love M, Hilbeck A, 2017. The distinct properties of natural and GM cry insecticidal proteins. Biotechnol Genet Eng Rev 33(1): 62-96. Mesnage R, Defarge N, Spiroux de Vendomois J, Seralini GE, 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem Toxicol 84: 133-153. Mesnage R, Renney G, Seralini GE, Ward M, Antoniou MN, 2017. Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. Sci Rep 7: 39328.]	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry_Labour/Soc.A/Health	II.1.4 Toxicology	AUT Comment_14	Additional remark In the Scientific Information, p. 59, the notifier states that the newly expressed proteins (CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1) have no synergistic or antagonistic effects to each other. But, this statement is not proven by toxicity studies: The notifier did not perform a whole food/feed study with GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. And the notifier did not perform a repeated-dose animal feeding study (28-day toxicity study) testing potential combinatory effects of individually expressed transgenic proteins in the GM maize stack. According to present knowledge, and generally stressed by notifiers of GM plants that produce Cry proteins, there exists a generalised mechanism for Bt toxins in relation to target organisms (lepidopteran insects). These similarities (e.g. the binding of midgut epithelial cells in insects, the development of membrane ion channels) give ample reason to assume that synergistic effects between single Cry proteins are rather likely than unlikely. Therefore, a proper assessment of health impacts in relation to simultaneous expression of different Bt toxins in GM maize and their presence in food and feed should be done. It should also be taken into consideration that Bt toxins can present different patterns of toxic response and can interact synergistically with chemicals such as pesticides (Grisolia et al. 2009). The notifier mentions the history of safe use of the newly introduced proteins in the GM stack MON87427xMON87460xMON89034x1507xMON87411x59122. However, the lack of general surveillance and consequently of exposure data and assessment of formerly authorised GM products means that there is no data whatsoever available on the consumption (and the safe use) of these GM plants and/or newly expressed proteins. In conclusion, there are considerable weaknesses in the assessment of the individual active principles. To strengthen the risk assessment, studies of effects of expressed Cry proteins and derived peptides on gastrointestinal microbiota or intestinal epithelial cells should be carried out as suggested in scientific literature (Farias et al. 2015). If animal studies are carried out to investigate the health impacts of simultaneous expression of Cry protein, 28-day repeated-dose toxicity studies with simultaneous application of purified proteins may be the best option.	The GMO Panel thanks Austria for the comment. As explained above, the GMO Panel did not consider necessary additional studies to investigate possible interactions of relevance for food and feed safety between the proteins newly expressed in this stack maize, taking into account their biological functions. The GMO Panel agrees with Austria's considerations on the history of safe use of these proteins.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				[Farias DF, Peijnenburg AA, Grossi-de-Sa MF, Carvalho AF, 2015. Food safety knowledge on the Bt mutant protein Cry8Ka5 employed in the development of coleopteran-resistant transgenic cotton plants. Bioengineered 6(6): 323-327. Grisolia CK, Oliveira R, Domingues I, Oliveira-Filho EC, Monerat RG, Soares AM, 2009. Genotoxic evaluation of different delta-endotoxins from Bacillus thuringiensis on zebrafish adults and development in early life stages. Mutat Res 672(2): 119-123.]	
Austria	Fed.Ministry _Labour/Soc .A/Health	II.1.5.2 Assessment of allergenicity of the whole genetically modified plant	AUT Comment_15	WHO gives information about existing food and inhalant maize allergens (WHO/IUIS 2015). However, because of lack of information about whole plant allergenicity studies with GM maize single events, it remains highly uncertain if genetic modification has affected overall allergenicity of the six single events and as a consequence the GM maize stacked event. The missing information should be provided. [WHO/IUIS, 2015. Allergen Nomenclature. Search Result "Zea mays (Maize)"; http://www.allergen.org/search.php?allergensource=maize&searchsource=Search; (last accessed: 28/08/2015).]	Maize is currently not considered a common allergenic food by European Regulation (Regulation 1169/2011). Therefore, an assessment based on specific experimental data targeting endogenous maize allergens is not currently requested by the EFSA GMO Panel. The GMO Panel has previously commented on the lack of experimental data specific for GM maize (EFSA, 2017). In such document, it was stressed that, the example provided on how to identify and select allergens, and interpret results for allergenic sources such as soybean described in the latest allergenicity guidance document of the GMO Panel (2017e) may be used for other crops than soybean in the future, if considered necessary. For these considerations, it was mentioned that risk assessors, risk managers, health professionals and stakeholders can provide valuable feedback. Based on these, the plants to be subject to the endogenous allergenicity assessment might be revised in the future.
Austria	Fed.Ministry _Labour/Soc .A/Health	II.5.3.2 Plant to micro-organisms gene transfer	AUT Comment_16	Scientific Information, p. 95 GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 is carrier of the antibiotic resistance marker gene nptII. By placing this transgenic variety on the market the applicant is violating Directive 2001/18/EC which requires a step-by-step phasing out of ARM genes in GMOs, which may have adverse effects on human health and the environment (Art. 4 (2) Dir. 2001/18/EC) by the end of 2004 (concerning GMOs released for market according to part C) and 2008 (concerning the deliberate release of GMOs into the environment according to part B of the directive) (EC 2001). Directive 2001/18/EC additionally stresses that "Member States and the Commission shall ensure that GMOs which contain genes expressing resistance to antibiotics in use for medical or veterinary treatment are taken into particular consideration when carrying out an environmental risk assessment " (EC 2001). NptII inactivates the critically important antibiotics kanamycin and neomycin which are in use for medical and veterinary	The GMO Panel took note of this comment. The applicant in accordance with Article 4(2) of Directive 2001/18/EC and with Reg (EU) 503/2013, should aim to develop GMOs without the use of antibiotic resistance marker genes. The antibiotic resistance traits as present in GM plants and/or their derived products are evaluated on a case-by-case basis with respect to their safety for humans, animals and the environment by the GMO

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				treatments in several European countries (Woegerbauer 2007; WHO 2012). EMEA is of the opinion that "These antibiotics are of importance for veterinary and human use, and their current or potential future use cannot be classified as of no or only minor therapeutic relevance." (EMEA 2007). Facing the current global crisis in antibiotic resistance (Neu 1992; French 2010; United Nations General Assembly 2016; WHO 2016; Martens and Demain 2017) it is irresponsible to fuel the environmental resistance gene pool with artificially and unnecessarily introduced variants of plant-derived nptII molecules and fragments thereof. EFSA BIOHAZ Panel Members state in their Minority Opinion on the issue that "it would be imprudent to regard resistance to any antibiotic as being of little or no relevance to human health " and that for characterising the risk of the transfer of plant-derived antibiotic resistance marker genes as "high, low or unlikely, one needs to be able to estimate probabilities of antibiotic gene transfer from GM plants to bacteria. These probabilities are below the detection limits for the studies reported " (EFSA 2009). Under special circumstances (i.e. low intrinsic nptII prevalence in environments exposed to transgenic maize DNA) nptII is acting as environmental pollutant (Woegerbauer et al. 2015c). NptII fulfils all requirements to be characterised as environmental pollutant according to Martinez, Pruden et al., and Keen et al. (Pruden et al. 2006; Keen and Montforts 2012; Martínez 2012): nptII is of anthropogenic origin (i.e. the transgenic insert cassette containing nptII was artificially assembled), was originally isolated from a mobile genetic element (Tn5) present on a pathogen, and anthropogenic actions (i.e. cultivation on agricultural fields) raise its concentration above the naturally occurring background levels. Cultivation of GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 on agricultural fields with nptII copy numbers below the limit of detection is therefore to be considered as deliberate contamination of natural environments with antibiotic resistance genes. Concerning the exposure of pathogens with nptII via transgenic GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 containing food or feed the situation is similar: the prevalence of (human) pathogens carrying nptII appears to be extremely low (Woegerbauer et al. 2014); any additional input of nptII copies into the system is, therefore, significant for the risk	Panel (see EFSA, 2009b). The GMO panel has already assessed the presence of the *nptII* gene in MON 87460 and no safety concerns were identified (EFSA GMO Panel, 2012; 2019b). The GMO Panel took note of this comment and reminds that the scope of this application is for import/processing for food/feed uses, excluding cultivation. Please refer to the clarifications provided in the frame of the previous comments and see also Section 6.1.1.2 of EFSA GMO Panel (2012) and Section 3.4.4.1 of EFSA GMO Panel (2019b).

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				assessment. By placing this product on the market the applicant acts again against the intention of Commission Implementing Regulation (EC) 503/13 which states in Recital 17 that "it is now possible to develop GMOs without the use of antibiotic resistance marker genes. Against this background and in accordance with Article 4(2) of Directive 2001/18/EC, the applicant should therefore aim to develop GMOs without the use of antibiotic resistance marker genes " (EC 2013). In this context it is noteworthy that the applicant has designed a genetic construct relying on the cre/lox system which would have allowed the excision of the nptII gene - which is flanked by loxP sites - from the plant genome (Monsanto 2010). The applicant has omitted to take advantage of this opportunity. In the adult transgenic plant nptII has no function and is, therefore, to be characterised as superfluous DNA. By placing this transgenic variety on the market the applicant is again violating Commission Implementing Regulation (EC) 503/13 which states that "the applicant shall endeavour to minimise the presence of inserted nucleic acid(s) sequences not essential to achieve the desired trait " (EC 2013). EFSA had noted a similar recommendation in its 2006 version of the guidance document for the risk assessment of genetically modified plants and derived food and feed (EFSA 2006). The risk characterisation of ARM genes in transgenic plants as performed by EFSA in its comprehensive 2009 approach is lacking quantitative data on resistance gene copy numbers homologous to plant-derived ARM genes in exposed environments (i.e. soil or the mammalian gastrointestinal tract). However, quantitative data are a prerequisite for adequately characterising a risk arising from transgenic gene transfers to bacterial populations (Woegerbauer 2007; Ma et al. 2011). Quantitative data have either been not available at all or the probabilities for ARM gene transfers were below the detection limits of the applied test systems in the reports considered for EFSA's Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants" (EFSA 2009). The EFSA GMO Panel concluded that adverse effects on	The GMO Panel took note of this comment. The GMO Panel took note of this comment.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				human and animal health and the environment resulting from the transfer of the nptII present in maize MON87460 to bacteria are unlikely and presented the following line of argumentation in support of its position (EFSA Opinion; Annex G; (EFSA 2012)): (1) The integration of the nptII gene through non-homologous recombination is most unlikely. (2) The stabilisation of the nptII gene into bacterial cells by double homologous recombination of A. tumefaciens sequences flanking the nptII gene, and subsequent dissemination in the environment are unlikely. (3) The unlikely but theoretically possible transfer of the nptII in maize MON 87460 to bacteria via gene replacement does not raise concerns due to the lack of an additional selective advantage which would be provided to the recipients in the receiving environments. We cannot concur with the conclusions of the EFSA GMO Panel and explain our position in the following section: Ad 1) The EFSA GMO Panel in its Scientific Opinion is neglecting the possibility and relevance of homology-facilitated illegitimate recombination. Although also considered as a rare event the frequency of HFIR is orders of magnitude higher compared to strictly non-homologous recombination. The frequency of illegitimate recombination in Acinetobacter baylyi was established to be approx. 0.01%, in Pseudomonas stutzeri ATCC17587 0.0003%, and in S. pneumoniae 0.9% compared to strictly homologous recombination (de Vries and Wackernagel 2004). These rates are in no way prohibitive for horizontal gene transfer and recombination events. Moreover, at the time of writing their opinion the EFSA GMO Panel (EFSA 2009) had only insufficient quantitative data available on the prevalence and actually occurring copy number of nptII genes in relevant natural environments (i.e. no quantitative real time PCR data from soil or gut environments) which may act as recombination partner molecules. However, quantitative data are necessary. The EFSA GMO Panel is promoting the notion that HGT processes within bacterial populations are orders of magnitude more frequent compared to the rate of gene transfer from plants to bacteria rendering the latter process irrelevant (EFSA 2009; EFSA 2012). Nevertheless free extracellular transgenic plant DNA becomes an interesting player concerning HGT in ecosystems with a low intrinsic prevalence of nptII carriers because plant DNA is continuously shed into the environment	The GMO Panel took note of these comments and is aware that in addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end joining and microhomology-mediated end joining are theoretically possible (Hülter and Wackernagel, 2008; EFSA, 2009). Synergistic effects of the recombinant genes, for instance due to combinations of recombinogenic sequences, which would cause an increase in the likelihood for HGT or a selective advantage were not identified. Therefore, the GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this six-event stack maize to bacteria does not raise any environmental safety concern. HGT of recombinant genes from GM plants to bacteria has never been shown under field conditions with GM plants used in agriculture. Moreover, the GMO Panel reminds that the scope of this application is for import/processing for food/feed uses, excluding cultivation.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				by root exudates, pollen and plant debris (de Vries et al. 2003; Woegerbauer et al. 2015d). Ad 2) The EFSA GMO Panel appears to be fixed on gene replacements by double crossovers as the only possible mechanism of relevance concerning the gene transfer from plant to bacteria. We would like to indicate that a) double homologous recombination is indeed the standard model for recombination presented in textbooks but certainly not the only recombination mechanism of relevance in naturally occurring bacterial populations, b) not only the transfer of whole intact genes is of relevance for the risk assessment of antibiotic resistance genes, but also the transfer fragments thereof creating mosaic sequence patterns as a result of recombination (Woegerbauer et al. 2015b), c) flanking A. tumefaciens sequences are no prerequisite for double homologous recombination. NptII sequences themselves can provide the substrate for double homologous recombination with endogenously present nptII homologs. The relevance of this process would be that mutations may be introduced into the resulting construct with the potential to change the substrate specificity of the newly expressed phosphotransferase enzyme (Woegerbauer et al. 2015b). It is not clear why – after genomic stabilisation – this nptII gene should not be disseminated in the environment. We would like to bring into mind that nptII is usually associated with transposon Tn5 in bacterial genomes (Beck et al. 1982). There is no convincing explanation available why this transposon should be inactivated if the nptII element recombines with incoming DNA. Ad 3) Kanamycin and neomycin are applied in animal husbandry - although with country-specific variations - to a substantial extent (Woegerbauer 2007). Manure used as fertilizer for fields intended as growing area for the present maize stack may provide substantial selection pressure for exposed plant-associated and soil bacteria (Chee-Sanford et al. 2009; Harms and Bauer 2012; Heuer et al. 2012; Casey et al. 2013; Joy et al. 2013; Marti et al. 2013; Peng et al. 2015; Woegerbauer et al. 2015d; Xiong et al. 2015; Widyasari-Mehta et al. 2016). Gut bacteria in neomycin/kanamycin treated animals may be exposed with nptII containing transgenic DNA via feed (Woegerbauer 2007). Considering the available data on aminoglycoside (neomycin/kanamycin) application in livestock and although there are still substantial knowledge gaps concerning the factors which may actually impose	The potential formation of mosaic genes is taken into account in the HGT assessment. The GMO Panel considers that non-homologous (illegitimate) recombination is possible but, in comparison with homologous recombination, does not contribute significantly to HGT events. In this case, natural variants of the bacterial genes exist in the environment and the likelihood of their HGT is much higher than for the transfer from GM plants to bacteria. The GMO Panel considered that if the *nptII* cassette from maize MON 87460 is transferred to bacterial cells, the expression of the gene cannot be excluded In EFSA GMO Panel (2012) is also stated that in case of substitution of a natural *nptII* gene by the *nptII* gene of maize MON 87460 this would not confer a novel trait, and thus not provide an additional selective advantage. The GMO Panel took note of this comment and reminds that the scope of this application is for import/processing for food/feed uses, excluding cultivation. The acquisition of the *nptII* gene by bacteria without *nptII* genes could confer resistance to kanamycin or neomycin, and thus provide a selective advantage in habitats in which these antibiotics would be present, i.e. the gastrointestinal tract of animals receiving kanamycin or neomycin orally (EFSA, 2009) has been specifically considered in the assessment of maize MON 87460. The analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses (EFSA GMO Panel, 2012).

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				selection of transgenic nptII recombinants it is more reasonable to assume the presence of selection pressure in environments exposed to this transgenic maize variety than denying it. The applicant is of the opinion that HGT of nptII does not offer an evolutionary advantage, because "the genes would have been transferred to other microbes during evolution via HGT from microbes already possessing this gene." We would like to indicate that the applicant is ignoring the potential for creating genetic variability by the transfer of mutated nptII gene variants or fragments thereof (Woegerbauer et al. 2015a). The transgenic nptII gene is affected by the same intrinsically active mutation rate as any other plant gene. If released into the environment by plant decay or root exudates, DNA is expected to get fragmented and suffer from lesions (Pontiroli et al. 2007; Pietramellara et al. 2009; Poté and Wildi 2012; Morrissey et al. 2015). Even DNA fragments and damaged DNA are taken up by competent bacteria leading to the formation of mosaic genes coding for proteins with new phenotypic properties (Woegerbauer et al. 2015b) or (if only short fragments are involved) are inducing mutations in the receiving genome (Overballe-Petersen et al. 2013). Furthermore we would like to stress that we have found a fully functional mosaic version of the nptII wild type gene in a pathogenic Pseudomonas aeruginosa strain which is carrier of a plasmid of environmental origin (Xiong et al. 2013; Woegerbauer et al. 2015a). The applicant is concluding that "it is clear that the occurrence of HGT from GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 to micro-organisms is extremely unlikely " and that "DNA transfer from GM plants to bacteria, if occurring, is considered to be of low frequency compared with gene transfer between bacteria." We would like to reiterate that nptII is of bacterial origin and was not plant codon optimised. Consequently, transgenic nptII represents an optimal partner molecule for recombination with similar chromosomally or episomally located homologous sequences in bacteria. Additionally, we must again point to the fact that especially concerning the fate of antibiotic resistance genes in bacterial populations frequency estimates of horizontal gene transfers are not predictive for (adverse) long-term effects (Pettersen et al. 2005). A single extremely rare event may easily be amplified and gain relevant proportions in the affected bacterial	The EFSA GMO Panel assessed in previous opinions the probability and potential adverse effects of HGT of the recombinant DNA for the single events (see Table 1 in the Scientific Opinion) including the case of MON 87460 (EFSA GMO Panel, 2012a; 2019b). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by HR. The GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this six-event stack maize to bacteria does not raise any environmental safety concern.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				population under appropriate selection pressure (Woegerbauer 2007; Nielsen et al. 2014). Concerning nptII all available data let it appear to be prudent to assume the presence of selection pressure in exposed environments supportive for the propagation of this resistance determinant (Chee-Sanford et al. 2009; Harms and Bauer 2012; Heuer et al. 2012; Casey et al. 2013; Joy et al. 2013; Marti et al. 2013; Peng et al. 2015; Woegerbauer et al. 2015d; Xiong et al. 2015; Widyasari-Mehta et al. 2016). [Beck E, Ludwig G, Auerswald EA, Reiss B, Schaller H, 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. Gene 19(3): 327-336. Casey JA, Curriero FC, Cosgrove SE, Nachman KE, Schwartz BS, 2013. High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant Staphylococcus aureus infection in Pennsylvania. JAMA Intern Med 173(21): 1980-1990. Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI, 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J Environ Qual 38(3): 1086-1108. de Vries J, Heine M, Harms K, Wackernagel W, 2003. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an Acinetobacter sp. Appl Environ Microbiol 69(8): 4455-4462. de Vries J, Wackernagel W, 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. Plant Soil 266(1-2): 91-104. EC, 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities. L 106: 1-38. EC, 2013. Commission Implementing Regulation (EU) No	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48. EFSA, 2006. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100. EFSA, 2009. Consolidated presentation of the joint scientific opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". The EFSA Journal 1108: 1-8. EFSA, 2012. Scientific Opinion on an application (EFSA-GMO-NL-2009-70) for the placing on the market of genetically modified drought tolerant maize MON 87460 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. Annex A + G. EFSA Journal 10(11): 2936. EMEA, 2007. Presence of the antibiotic resistance marker gene nptII in GM plants for food and feed uses. EMEA/CVMP/56937/2007. French GL, 2010. The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 36 Suppl 3: S3-7. Harms K, Bauer J, 2012. Detection and occurrence of antibiotics and their metabolites in pig manure in Bavaria (Germany). Antimicrobial resistance in the environment. Keen, P. L., Montforts, M. H. M. M. Hoboken, New Jersey, USA, Wiley & Sons Inc.: 293 - 307. Heuer H, Kopmann C, Zimmerling U, Krögerrecklenfort E, Kleineidam K, Schloter M, Top E, Smalla K, 2012. Effect of veterinary medicines introduced via manure into soil on the	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				abundance and diversity of antibiotic resistance genes on their transferabilty. Antimicrobial resistance in the environment. Keen, P. L., Montforts, M. H. M. M. Hoboken, New Jersey, Wiley & Sons, Inc.: 453-463. Joy SR, Bartelt-Hunt SL, Snow DD, Gilley JE, Woodbury BL, Parker DB, Marx DB, Li X, 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environ Sci Technol 47(21): 12081-12088. Keen PL, Montforts MHMM, 2012. Antimicrobial resistance in the environment, Wiley-Blackwell. Ma BL, Blackshaw RE, Roy J, He T, 2011. Investigation on gene transfer from genetically modified corn (Zea mays L.) plants to soil bacteria. J Environ Sci Health B 46(7): 590-599. Martens E, Demain AL, 2017. The antibiotic resistance crisis, with a focus on the United States. J Antibiot (Tokyo). Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Topp E, 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Appl Environ Microbiol 79(18): 5701-5709. Martínez JL, 2012. Natural antibiotic resistance and contamination by antibiotic resistance determinants: The two ages in the evolution of resistance to antimicrobials. Frontiers in Microbiology 3(JAN). Monsanto, 2010. Application for authorization to place on the market MON 87460 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed. Technical Dossier. Part I. Morrissey EM, McHugh TA, Preteska L, Hayer M, Dijkstra P, Hungate BA, Schwartz E, 2015. Dynamics of extracellular DNA decomposition and bacterial community composition in soil. Soil Biol Biochem 86: 42-49. Neu HC, 1992. The crisis in antibiotic resistance. Science	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
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Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
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Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Fuchs K, Nielsen KM, Allerberger F, 2014. Prevalence of the aminoglycoside phosphotransferase genes aph(3')-IIIa and aph(3')-IIa in Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica and Staphylococcus aureus isolates in Austria. J Med Microbiol 63(Pt 2): 210-217. Woegerbauer M, Zeinzinger J, Steinwider J, Axmann S, Dominques S, Fuchs R, Gottsberger R, Hofrichter J, Hufnagl P, Indra A, Kopacka I, Kornschober C, Korschineck I, Pascher K, Reisenzein H, Schleicher C, Schwarz M, Springer B, Allerberger F, Nielsen KM, Fuchs K, 2015d. Baseline prevalence of neomycin phosphotransferase genes II and III in maize and potato fields, feed and human bacterial pathogens in Austria. Federal Ministry of Health: 1-387. Xiong J, Alexander DC, Ma JH, Deraspe M, Low DE, Jamieson FB, Roy PH, 2013. Complete sequence of pOZ176, a 500-kilobase IncP-2 plasmid encoding IMP-9-mediated carbapenem resistance, from outbreak isolate Pseudomonas aeruginosa 96. Antimicrob Agents Chemother 57(8): 3775-3782. Xiong W, Sun Y, Ding X, Wang M, Zeng Z, 2015. Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. Frontiers in Microbiology 6(MAR).]	
Austria	Fed.Ministry _Labour/Soc .A/Health	II.5.3.2 Plant to micro-organisms gene transfer	AUT Comment_17	Scientific Information, p. 97 The applicant maintains that "current scientific evidence indicates that the transfer of genes derived from GM plants into bacteria and their stable integration, either does not occur or, unlikely, it has been below the limit of detection in all the studies performed." We would like to point to the fact that - quite to the contrary - it is highly likely that the studies which analysed the frequency of horizontal gene transfer from plant to bacteria and which retrieved negative results were affected by insufficient detection limits (Heinemann and Traavik 2004; Nielsen and Townsend 2004; Townsend et al. 2012; Nielsen et al. 2014). The word "unlikely" in this context is misleading and highly inappropriate according to recent literature. We would like to ask the EFSA GMO panel to take note of it. The applicant maintains that a "widespread occurrence of resistance in bacterial populations" would render the risk of	The GMO Panel took note and thanks Austria for this and other comments on the potential risk associated with horizontal gene transfer.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				nptII transfer from GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 to bacteria insignificant. We would like to call attention to the fact that the risk of the resistance gene is to be assessed. Therefore, the number of introduced transgenic nptII genes has to be evaluated against the nptII counterparts already endogenously present in receptor bacteria in exposed populations (Demanèche et al. 2008; Ma et al. 2011; Woegerbauer et al. 2015). In fact the prevalence and quantity of nptII genes in relevant natural environments are extremely low (Woegerbauer et al. 2015). Any artificial input of nptII copies into these ecosystems is therefore to be considered as relevant for the risk assessment. Transgenic maize plants are supplementing the pool of free extracellular DNA in soil and are therefore - indeed not the only but nevertheless - important players in the field. Due to the high complexity and indeterminate nature of the system guarding the evolution and dissemination of antibiotic resistance genes in natural environments and their eventual transfer to clinically relevant pathogens (Manaia 2017) it would be fair to admit that there are still significant knowledge gaps which do not allow a comprehensive and convincing risk assessment of plant-derived transgenic nptII and which make it impossible to draw meaningful conclusions with a sufficiently high certainty. Facing the actual crisis in antibiotic resistance it would wise to implement the precautionary principle, especially in the present case where the application of nptII as a marker gene was completely unnecessary and the removal of the nptII from the plant genome was possible. The technology is outdated and is practically legally banned from application already for years - nevertheless lifetime and worldwide exposure to this antibiotic resistance gene is to be expected. Taken together all these facts may render even the most remote possibility for adverse effects as inacceptable for the community (Rajan and Letourneau 2012). We would like to ask the GMO Panel to take note of these considerations. [Demanèche S, Sanguin H, Poté J, Navarro E, Bernillon D, Mavingui P, Wildi W, Vogel TM, Simonet P, 2008. Antibiotic-resistant soil bacteria in transgenic plant fields. Proc Natl Acad Sci U S A 105(10): 3957-3962. Heinemann JA, Traavik T, 2004. Problems in monitoring	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				horizontal gene transfer in field trials of transgenic plants. Nat Biotechnol 22(9): 1105-1109. Ma BL, Blackshaw RE, Roy J, He T, 2011. Investigation on gene transfer from genetically modified corn (Zea mays L.) plants to soil bacteria. J Environ Sci Health B 46(7): 590-599. Manaia CM, 2017. Assessing the Risk of Antibiotic Resistance Transmission from the Environment to Humans: Non-Direct Proportionality between Abundance and Risk. Trends Microbiol 25(3): 173-181. Nielsen KM, Bohn T, Townsend JP, 2014. Detecting rare gene transfer events in bacterial populations. Front Microbiol 4: 415. Nielsen KM, Townsend JP, 2004. Monitoring and modeling horizontal gene transfer. Nat Biotechnol 22(9): 1110-1114. Rajan SR, Letourneau DK, 2012. What risk assessments of genetically modified organisms can learn from institutional analyses of public health risks. J Biomed Biotechnol 2012: 203093. Townsend JP, Bohn T, Nielsen KM, 2012. Assessing the probability of detection of horizontal gene transfer events in bacterial populations. Front Microbiol 3: 27. Woegerbauer M, Zeinzinger J, Steinwider J, Axmann S, Dominques S, Fuchs R, Gottsberger R, Hofrichter J, Hufnagl P, Indra A, Kopacka I, Kornschober C, Korschineck I, Pascher K, Reisenzein H, Schleicher C, Schwarz M, Springer B, Allerberger F, Nielsen KM, Fuchs K, 2015. Baseline prevalence of neomycin phosphotransferase genes II and III in maize and potato fields, feed and human bacterial pathogens in Austria. Federal Ministry of Health: 1-387.]	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.5.3.2 Plant to micro-organisms gene transfer	AUT Comment_18	3.2.2. Step 2: Hazard characterisation Scientific Information, p. 99 The applicant maintains that "there is negligible potential for recombination between genetic material inherited in GM maize MON87427 x MON87460 x MON89034 x 1507 x MON87411 x 59122 and environmental prokaryotic micro-organisms due to limited bacterially derived sequence content, the sequence source, the organization of those bacterially derived sequences in GM maize MON87427 x MON87460 x MON89034 x 1507 x MON87411 x 59122 and the absolute requirement of the presence of a homologous sequence in the acceptor prokaryotic micro-organism." We would like to indicate that transformation of bacteria with prokaryotic elements embedded in plant genomic DNA is observable and no argument against successful recombination (Gebhard and Smalla 1998; Gebhard and Smalla 1999). And postulating an "absolute" requirement for the presence of homologous sequences is misleading: the rate of homologous recombination is decreasing in a log-linear relationship with increasing sequence divergence among the involved DNA molecules and fall below the level of detection at a sequence divergence above 25-30% (Fraser et al. 2007; Woegerbauer et al. 2015). We would like to ask the EFSA GMO Panel to take this into consideration. [Fraser C, Hanage WP, Spratt BG, 2007. Recombination and the nature of bacterial speciation. Science 315(5811): 476-480. Gebhard F, Smalla K, 1998. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol 64(4): 1550-1554. Gebhard F, Smalla K, 1999. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol Ecol 28(3): 261-272. Woegerbauer M, Kuffner M, Kopacka I, Domingues S, Steinwider J, Nielsen KM, Fuchs K, 2015. Impact of mosaic genes on the risk assessment of GMOs. Federal Ministry of Health: 1-268.]	The GMO Panel took this comment into consideration.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	II.5.3.2 Plant to micro-organisms gene transfer	AUT Comment_19	3.2.3. Step 3: Exposure characterisation Scientific Information, p. 100 The applicant describes a study by Gulden et al. and points out that the study authors did not observe an accumulation of transgenic plant DNA in the tested soil but forgets to mention that several samples tested positive for transgenic CP4 epsps even two years after the last transgenic crop was planted in the respective plot (Gulden et al. 2008). We would like to ask the EFSA GMO Panel to take care for a correct and full presentation of literature data by the applicants. Scientific Information, p. 101 The applicant maintains that "after duodenum passage, over 95% of DNA is hydrolyzed and bases are absorbed into the enterocytes." Considering a per capita uptake of transgenic inserts of 9 x 10E9 molecules per day of a genetically modified maize variety (Jonas et al. 2001) a reduction by 95% would mean that still approximately 1 x 10E7 intact molecules would be available in the system for bacterial transformation. A reduction by 95% is irrelevant concerning the risk assessment of transgenic inserts in relation to bacterial transformation. We would like to ask the EFSA GMO Panel to take note of this calculation. [Gulden RH, Lerat S, Blackshaw RE, Powell JR, Levy-Booth DJ, Dunfield KE, Trevors JT, Pauls KP, Klironomos JN, Swanton CJ, 2008. Factors Affecting the Presence and Persistence of Plant DNA in the Soil Environment in Corn and Soybean Rotations. Weed Sci 56: 767-774. Jonas DA, Elmadfa I, Engel KH, Heller KJ, Kozianowski G, Konig A, Muller D, Narbonne JF, Wackernagel W, Kleiner J, 2001. Safety considerations of DNA in food. Ann Nutr Metab 45(6): 235-254.]	The GMO Panel also took note of the comment and of the proposed calculation.
Austria	Fed.Ministry _Labour/Soc .A/Health	II.5.3.4 Interactions of the GM plant with non-target organisms (NTOs)	AUT Comment_20	The assessment of this area of risk by the notifier is considered insufficient due to the following reasons: - The notifier states that the ERA will primarily focus on indirect exposure to GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. However, only effects resulting from the newly expressed proteins are discussed and exposure pathways and potential effects resulting from the dsRNA on NTOs are - except for a reference to the agronomic assessment - not addressed in the	Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 grains is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the six-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins will not alter this conclusion.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				in-field assessment (Scientific Information, p. 108). A recent literature review of baseline information on RNAi identified substantial knowledge gaps regarding exposure, specificity, off-target effects, sequence similarities and bioinformatics (Christiaens et al. 2018). In particular, there are insufficient scientific data available regarding indirect exposure to dsRNA. However, such exposure pathways are relevant for the import and use of GM MON87427xMON87460xMON89034x1507xMON87411x59122. - The hazard characterisation is based on the current concept of the mode of action of Cry toxins exerting toxicity via binding to specific receptors. However, recent data indicate that certain aquatic organisms can be affected by Cry toxins despite lacking respective receptors (for review see (Venter and Bøhn 2016)) and that Cry toxins are less specific than previously assumed (van Frankenhuyzen 2013; Hilbeck and Otto 2015). For example, negative fitness effects have been shown for Daphnia magna, a non-target model organism, under chronic dietary exposure to kernels (Bøhn et al. 2008) and leaves of Bt maize (Holderbaum et al. 2015). Similar effects were recently found during tests conducted with purified Cry proteins and determined to be dose-dependent (Bøhn et al. 2016). This study also indicated combinatorial effects between different Cry proteins as well as between Cry proteins and Glyphosate-based herbicides indicating that stronger effects may be expected for stacked event GMPs, like GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. In our opinion, the notifier does not adequately assess the potential eco-toxicological effects on the basis of experimental data, in particular chronic effects on selected focal species (e.g. water and soil organisms most likely exposed to faeces and manure from animals fed with GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. In our view, potential effects resulting from the dsRNA and siRNAs are not adequately taken into account and are associated with substantial uncertainties (EFSA 2014; EPA 2014). Thus, the presented exposure and hazard assessments are not sufficiently robust and should be revised by the notifier. [Bøhn T, Primicerio R, Hessen DO, Traavik T, 2008. Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety. Arch Environ Contam Toxicol 55(4): 584-592.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Bøhn T, Rover CM, Semenchuk PR, 2016. Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food Chem Toxicol 91: 130-140. Christiaens O, Dzhambazova T, Kostov K, Arpaia S, Joga MR, Urru I, Sweet J, Smagghe G, 2018. Literature review of baseline information on RNAi to support the environmental risk assessment of RNAi-based GM plants. EFSA Supporting Publications 15(5): 1424E. EFSA, 2014. International scientific workshop 'Risk assessment considerations for RNAi-based GM plants' (4-5 June 2014, Brussels, Belgium). EFSA Supporting publication. 2014:EN-705. EPA, 2014. RNAi Technology: Program Formulation for Human Health and Ecological Risk Assessment - FIFRA Scientific Advisory Panel Meeting, January 28th, 2014. SAP Minutes No. 2014-02, 1-75. Hilbeck A, Otto M, 2015. Specificity and combinatorial effects of Bacillus thuringiensis Cry toxins in the context of GMO environmental risk assessment. Frontiers in Environmental Science 3(71): 1-18. Holderbaum DF, Cuhra M, Wickson F, Orth AI, Nodari RO, Bøhn T, 2015. Chronic responses of Daphnia magna under dietary exposure to leaves of a transgenic (event MON810) Bt-maize hybrid and its conventional near-isoline. Journal of Toxicology and Environmental Health - Part A: Current Issues 78(15): 993-1007. van Frankenhuyzen K, 2013. Cross-order and cross-phylum activity of Bacillus thuringiensis pesticidal proteins. J Invertebr Pathol 114(1): 76-85. Venter HJ, Bøhn T, 2016. Interactions between Bt crops and aquatic ecosystems: A review. Environ Toxicol Chem 35(12): 2891-2902.]	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry_Labour/Soc.A/Health	II.6 Post-Market Environmental Monitoring Plan (PMEM)	AUT Comment_21	6.1 General The monitoring plan presented is very general and basically identical to monitoring plans for other GM maize products submitted previously. Previous recommendations and suggestions for improvements submitted by Austria - based on issues discussed in the scientific literature, in scientific reports of competent authorities from various member states (see e.g. (Züghart et al. 2011)) or derived from the review of monitoring approaches for other GM maize lines by EFSA (e.g. (EFSA 2011b; EFSA 2012; EFSA 2013)) - were not taken into account. In particular, the notifier does not specifically consider the potential exposure of EU environments to GM maize MON87427xMON87460xMON89034x1507xMON87411x59122 other than by unintended release of substantial volumes of viable GM maize grain via losses during loading or unloading for processing into animal feed or human food products. This is in contrast to the ERA which includes indirect exposure pathways resulting from the use of the GM maize stack (Scientific Information, p. 89). Consequently, exposure via waste materials from processing or use should particularly be considered in accordance with current EFSA guidance (EFSA 2011a). Since all exposure pathways should be taken into account in the monitoring plan, we consider the monitoring plan at hands to be insufficient to address the potential environmental effects of GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. In our view, the monitoring plan at hand does not ensure that relevant information for the monitoring of the product is gathered and therefore cannot be considered adequate, but needs to be improved. [EFSA, 2011a. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316: 1-40. EFSA, 2011b. Scientific Opinion of the GMO Panel on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. The EFSA Journal 9(10):2376: 1-66. EFSA, 2012. Scientific Opinion of the GMO Panel on the annual Post-Market Environmental Monitoring (PMEM) report from	Monitoring and its practical implementation are related to risk management, and thus a final adoption of the post-market environmental monitoring plan falls outside the mandate of EFSA.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. The EFSA Journal 10(4):2610:1-35. EFSA, 2013. Scientific Opinion of the GMO Panel on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2011. The EFSA Journal 11(12):3500: 1-38. Züghart W, Raps A, Wust-Saucy A-G, Dolezel M, Eckerstorfer M, 2011. Monitoring of genetically modified organisms. A policy paper representing the view of the National Environment Agencies in Austria and Switzerland and the Federal Agency for Nature Conservation in Germany. Umweltbundesamt Wien, Reports, Volume 0305, ISBN: 978-3-99004-107-9; http://www.umweltbundesamt.at/aktuell/publikationen/publikationssuche/publikationsdetail/?pub_id=1903.]	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry_Labour/Soc.A/Health	II.6.3 General Surveillance (strategy, method)	AUT Comment_22	The basis for the ERA presented by the notifier is associated with a number of shortcomings (see comments to sections 1.2, 1.3 and 1.4) and thus uncertainties remain regarding the environmental risk associated with GM maize MON87427xMON87460xMON89034x1507xMON87411x59122. The proposed general surveillance for unanticipated adverse is not sufficiently elaborated and should be amended regarding the following elements: • Elaboration of a detailed monitoring methodology (e.g. parameters, specific information). • Identification of existing national institutions and operators involved in GS in individual Member States and evidence for their commitment to GS activities. • Assignment of clear responsibilities and concrete tasks to each party involved. • Verification of the skills and expertise of the parties involved which are required for the detection of potential adverse environmental impacts. • Taking into account all potential routes of exposure under commercial use, a fundamental requirement of the EU-approach to monitoring (EFSA 2011). (Involvement of operators further down the food and feed chain, e.g. veterinary networks). • Specification of the specific measures based on HACCP principles in order to verify whether they match with the requirements of environmental monitoring. • More specific data on transport and handling of GM maize grain (e.g. actual import volumes, transport routes, processing plants, amounts used for feed) in order to provide a basis for the development and implementation of national monitoring concepts. [EFSA, 2011. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316: 1-40.]	Monitoring and its practical implementation are related to risk management, and thus a final adoption of the post-market environmental monitoring plan falls outside the mandate of EFSA.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Austria	Fed.Ministry _Labour/Soc .A/Health	Part I – General information	AUT Comment_01	I. GENERAL REMARKS The notifier considers the whole notification documents as confidential business information, even the "Scientific Information". EFSA has stated that confidential business information should be highlighted in the following way: "FROM CBI: Smith et al. 2003". Therefore, we must point out that the whole Austrian statement refers to information considered as confidential business information by the notifier (i.e. all comments submitted via EFSA DMS System).	The GMO Panel took note of the information.
Austria	Fed.Ministry _Labour/Soc .A/Health	Part V - Methods of detection, sampling and identification and reference material	AUT Comment_23	Detection method Providing an event specific detection method for each parental line and a specific reference PCR system is not satisfactory. Generally, a validated event specific detection method for the stacked event should be presented before deciding about the placing on the market of this product. Furthermore, as long as no official (guidance) document on the interpretation of detection results, i.e. how to distinguish between a stacked event and its respective single events, of the described method for stacked events is available, no approval for placing on the market of this product should be given. The detection method as presented by the notifier was submitted to EU-RL GMFF for validation purposes. The current evaluation status of the method is "Step 5 (Reporting)" (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx).	This is beyond the remit of EFSA. (The EURL GMFF is responsible for the method validation.)
Belgium	Biosafety Advisory Council	II.1.2.2 Information relating to the genetically modified plant	Comment from Belgium	From the expression levels of the different genes in the stacked event described in Tables 1-11 it can be observed that for most of the genes the expression levels are higher than in the single events used as a control. In relation to the variable expression levels for the CP4 EPSPS gene, that is introduced twice, the applicants refer to the scientific literature to explain that expression and silencing patterns among homologous genes is a natural phenomenon in plants and may result in up- and down-regulation of gene expression which can even be tissue dependent. For the genes that are only introduced in one copy, e.g. PAT, it is not clear if the difference is statistically significant. Nevertheless, this does not raise safety issues.	The EFSA GMO panel takes note of the comment.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.1 Hazard identification and characterisation	Comment 1/ Federal Agency for Nature Conservation (BfN)	The Federal Agency for Nature Conservation (BfN) considers that further information should be presented before the risk assessment of EFSA/GMO/NL/2017/139 can be finalised. Agronomic data should be amended and include data on the occurrence of volunteers. Although „MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122" (in the following referred to as "the GMO") is not intended for cultivation the BfN strongly suggests that the applicant provides detailed information on the wild relative teosinte, which has been found repeatedly in EU fields. Spillage of maize seed during transport must be anticipated, hence introgression of the insect resistance (IR) trait into teosinte should be considered. IR may increase fitness in teosinte and thus the likelihood of the plant to become invasive and/or a pest problem. As quantitative data on the fate of Bt proteins from feed and processing are missing the BfN recommends to generate such data in order to be able to calculate their environmental concentration and to assess the potential risk for non-target organisms. In addition, the present monitoring plan does not comply with Directive 2001/18/EC and thus needs to be amended.	The information provided by the applicant in application EFSA-GMO-NL-2017-139 was sufficient to conduct the environmental risk assessment of the GM maize. Monitoring and its practical implementation are related to risk management, and thus a final adoption of the post-market environmental monitoring plan falls outside the mandate of EFSA.
Germany	BfN	II.1.2.2 Information relating to the genetically modified plant	Comment 2/ Federal Agency for Nature Conservation (BfN)	From CBI: The applicant investigates the potential for the DvSnf7 transcript produced in maize plants carrying the event MON87411, which forms a double stranded RNA hairpin structure, to target endogenous maize transcripts. The applicant identifies 26 potential off-targets and discards the possibility of an effect due to the presence of multiple mismatches between the DvSnf7 and the target sequence (2 or more). In contrast to the suggestions of EFSA in Annex II of the minutes of the 118th GMO plenary meeting, the applicant does not discuss "the established or predicted function of the potential off-targets that could impact on the safety of the GM plant and/or derived products as food/feed, or in the environment." In plants, trans-acting small non-coding RNAs derived from hairpin structures can mediate gene regulation through methylation by targeting regulatory sequences outside the transcriptome (Paces et al. 2017; Tarutani et al. 2010; Wu et al. 2010). The presented study is unsuited to exclude whether the DNA-dependent RNA polymerase II (Pol II) transcribed product of the transgenic DvSnf7 construct is influencing gene	The EFSA GMO panel requested additional information regarding off target effects of DvSnf7 at its clock 5, in compliance to the "Internal note on the strategy and technical aspects for small RNA plan off-target bioinformatics studies" (published with the minutes of 118th Panel meeting -Annex II). The information submitted confirmed previous results from the assessment of the single event, that do not raise any safety concerns.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				expression of endogenous maize genes as it only takes into account the maize transcriptome and not all potential gene regulatory sequence. In order to answer this question the applicant could alternatively identify and characterize whether and which small RNAs are produced in planta from the DvSnf7 construct in the event MON87411 and subsequently search the whole genomes of the respective maize accessions (LH244 and LH287) for putative off-target sites. References: Paces, Jan; Nic, Miloslav; Novotny, Tomas; Svoboda, Petr (2017): Literature review of baseline information to support the risk assessment of RNAi-based GM plants. In: EFSA Supporting publication 14 (6), e391. DOI: 10.2903/sp.efsa.2017.EN-1246. Tarutani, Yoshiaki; Shiba, Hiroshi; Iwano, Megumi; Kakizaki, Tomohiro; Suzuki, Go; Watanabe, Masao et al. (2010): Trans-acting small RNA determines dominance relationships in Brassica self-incompatibility. In: Nature 466 (7309), S. 983–986. DOI: 10.1038/nature09308. Wu, Liang; Zhou, Huanyu; Zhang, Qingqing; Zhang, Jianguang; Ni, Fangrui; Liu, Chang; Qi, Yijun (2010): DNA methylation mediated by a microRNA pathway. In: Molecular cell 38 (3), S. 465–475. DOI: 10.1016/j.molcel.2010.03.008.	
				The bioinformatics assessment of the introduced genetic elements returned pairwise qualifying alignments of the event MON 87460 with the Ti plasmid of Agrobacterium tumefaciens. Homologous recombination between these sites would replace a sequence with two "hypothetical proteins" from the plasmid of Agrobacterium tumefaciens with sequences from the event MON 87460 containing the nptII gene in control of the plant specific promoter 35S. NptII not only provides resistance for kanamycin but also for neomycin, geneticin (G418), gentamicin A/B, paromomycin, and framycetin. Kanamycin and neomycin are categorised as highly important antimicrobials by WHO (2007) and EMEA (2007). Agrobacterium tumefaciens is a tumor inducing plant pest with an extensive host range which can live in the soil over long periods. It is widely used in biotechnology for its ability to stable integrate genetic material into plant genomes. Heuer et al. (2011) found that "manure has become a reservoir of resistant bacteria and antibiotic compounds, and its application to agricultural soils is assumed to significantly increase antibiotic resistance genes and selection of resistant bacterial populations in soil… The human exposure to soil-	As reported in Section 6.1.1.2 of the EFSA GMO Panel Scientific opinion on MON87460 (EFSA GMO Panel, 2012) and Section 3.4.4.1 of the Scientific Opinion on MON 87427 × MON 87460 × MON 89034 × MIR162 × NK603 (EFSA GMO Panel, 2019b), three different scenarios of integration of the transgenes of maize MON 87460 to bacteria in the environment were considered. 1. Mobilisation of *nptII* by the cre/lox system. The GMO Panel considered that the stabilisation of the *loxP-nptII-loxP* fragment due to the Cre recombination system present in bacteria containing a P1 or P1-like bacteriophage was unlikely. 2. Transfer of *nptII* by double homologous recombination to a Ti-plasmid of *A. tumefaciens*. In EFSA GMO Panel (2012) is also recognised that the acquisition of the *nptII* gene by bacteria without *nptII* genes could confer resistance to kanamycin or neomycin, and thus provide a selective advantage in habitats in which these antibiotics would be present. The updated bioinformatic analysis for MON 87460 did not result in new information which would change previous conclusions on possible HGT. It was confirmed the possibility for a facilitated double homologous recombination between the T-tr7

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				borne resistance has yet to be determined, but is likely to be severely underestimated." The nptII gene in the event MON 87460 would unnecessarily add a further potential source for antibiotic resistance to the described selective environment in agricultural soil. Therefore, taking into account the two exposure routes, human and animal digestive systems and manure application on agricultural fields, further spreading of the antibiotic resistant marker gene via an EU wide marketed product should – in accordance with the precautionary approach and with Directive 2001/18/EC – be prevented. References: EMEA, Committee for Medicinal Products for Veterinary Use and Committee for Medicinal Products for Human Use, (2007): Presence of the Antibiotic Resistance Marker Gene nptII in GM Plants for Food and Feed Uses, EMEA/CVMP/56937/2007. World Health Organisation, 2007: Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to contain Antimicrobial Resistance due to Non-Human Antimicrobial Use, Report of the Second WHO Expert Meeting Copenhagen, 29–31 May 2007. Heuer, H., Schmitt, H., Smalla, K. (2011): Antibiotic resistance gene spread due to manure application on agricultural fields. Current Opinion in Microbiology 14, pp. 236-243. The applicant should be asked to explain the nearly twofold higher values for expression of Cry1A.105 in the stacked event in comparison to MON 89034. Since cross reactivity of the ELISA method for Cry1A.105 and the protein Cry1F was described in another application (Part II Scientific Information of Application for authorisation in the European Union of MON 89034 × 1507 × NK603 × DAS-40278-9; EFSA-GMO-NL-2013-112) the applicant should be asked to elaborate on that issue and, if necessary, to adjust the procedure to produce flawless expression data.	intervening sequence and the left border of the Ti cassette and the corresponding sequences in the *A. tumefaciens* Ti-plasmid downstream resulting in the insertion of the *nptII* expression cassette (*P35S/nptII/T-nos*). However, this led to the concomitant loss of a naturally occurring sequence in the *A. tumefaciens* Ti-plasmid resulting in a Ti-plasmid that would not promote for plant tumor formation (EFSA GMO Panel, 2012a). Due to the selective disadvantage of such bacterial recipients for growing in plants, and the natural abundance of *nptII* genes in the environmental bacterial communities, the GMO Panel concludes that there was no indication for a risk to human or animal health or to the environment. 3. Substitutive homologous recombination of *nptII* or *cspB* genes to the bacteria harbouring natural variants of such genes. The GMO Panel considered that if the *nptII* cassette from maize MON 87460 is transferred to bacterial cells, the expression of the gene cannot be excluded. In EFSA GMO Panel (2012a) is also stated that in case of substitution of a natural *nptII* gene by the *nptII* gene of maize MON 87460 this would not confer a novel trait, and thus not provide an additional selective advantage. The updated bioinformatic analysis for MON 87460 (study REG-2020-0212 submitted to EFSA on the 29 May 2020) shows that there is no sufficient sequence identity and length of the codon-optimised cspB gene from B. subtilis with bacterial DNA for homologous recombination. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses. The GMO Panel took note of this comment. The applicant in accordance with Article 4(2) of Directive 2001/18/EC and with Reg (EU) 503/2013, should aim to develop GMOs without the use of antibiotic resistance marker genes. The EFSA GMO panel requested clarifications to the applicant regarding protein expression during clocks 5 (19/01/2019), 8 (27/08/2019) and 10 (17/12/2019). The information has been assessed and included in the scientific opinion. All values are acceptable and in line with Commission Implementing Regulation (EU) No 503/2013.
Germany	BfN	II.1.3.1 Choice of the conventional	Comment 3/ Federal Agency for	It is acknowledged that the plant material used for comparative assessment including the GMO and the comparator were tested, but not the reference lines. However,	The information provided by the applicant in application EFSA-GMO-NL-2017-139 was considered sufficient but the GMO Panel requested the applicant provided further information (additional

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		counterpart and additional comparators	Nature Conservation (BfN)	test conditions, results and their evaluation are missing and tests for absence of other GM events in the control were not performed. To proof the purity of the control and comparator, starting seed corresponding results should be requested (cf. EFSA's request from 06.06.2016 for application EFSA-GMO-NL-2015-126 to provide purity levels for analysed seeds).	information received on 3/9/2018) to characterise the germinability of the starting materials (GM and comparator).
Germany	BfN	II.1.3.4 Comparative analysis of composition	Comment 4/ Federal Agency for Nature Conservation (BfN)	The applicant concludes that the GMO is compositionally similar to the conventional maize comparator and that the genetic modification is not a significant contributor to compositional variability in maize. However, given that 41% of the endpoints selected and analysed by the applicant are significantly different, substantial equivalence of the untreated GMO to the comparator should be questioned. Since the intended herbicides will extensively be used in commercial field production of the GMO, the effects of glyphosate and glufosinate on the plants metabolism have to be taken into account. In grain from the GMO treated with the intended herbicides mean levels of protein and all analysed amino acids are at least in the upper range or above the values of the references and consistently above the mean values of the comparator. Except for phosphorus, all minerals are significantly different. Overall the treated GMO is significantly different to the comparator in 63% of the assessed endpoints in the comparative analysis of composition with 8 endpoints "more likely than not" or non-equivalent to the reference varieties. Even though effects on food and feed safety might be considered unlikely, the observed differences indicate physiological changes in the GMO which might lead to increased susceptibility against stressors and thus, under certain environmental conditions, would require compensation by further plant protection measures. However, differences in composition are only – if at all – discussed regarding biological relevance from a food and feed safety perspective and the phenotypic parameters assessed do not allow for approximations regarding e.g. stress tolerance of the GMO. Furthermore, the applicant avoids discussion on biological relevance of the observed differences by citing ranges of extreme values from the ILSI Crop Composition Database (including data obtained over two decades by laboratories all over the world using a variety of different methods) to classify values as "within the natural variability". According to EFSA 2011 "the test of difference is used to verify	The GMO Panel assessed all significant differences between the six-event stack maize and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM maize reference varieties. None of the differences identified in forage and grain composition between the six event stack maize and the non-GM comparator needed further food/feed safety assessment except for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. The relevance of these changes was further discussed in Section 3.4.3.

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				whether the GM plant, apart from the introduced genetic modification(s), is different from the comparators(s) and therefore has the potential to cause adverse effects." The applicant should be asked to further assess the differences observed between composition of GMO, comparator and reference lines and to elaborate on the biological relevance of these differences to be able to exclude potential adverse effects of the genetic modification. The BfN recommends providing data on the amount of residual herbicides. Such data would complement the assessment of food and feed safety. References: EFSA (2011). Scientific opinion. Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 9(5): 2150, 5.	The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel.
Germany	BfN	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	Comment 5/ Federal Agency for Nature Conservation (BfN)	The event MON87460 in the stacked GMO under assessment has been engineered to differ in agronomic aspects in comparison to conventional maize. Even though no difference to the conventional counterpart could be observed in the comparative analyses, the intended resistance to abiotic stresses can be expected to change the plants phenotypic characteristics. The selected parameters for phenotypic characterization cannot sufficiently indicate differences in reproduction, dissemination, and survivability of the GMO compared to conventional maize. Increased fitness of maize would be most relevant in areas not under direct cultivation such as fallows, field strips or roadsides. Phenotypic data was obtained from GMO treated with pesticides. However, the use of agrochemicals may impact fitness and reduce stress such as competition or herbivory. The current test design therefore is not appropriate for comparative investigations of GMO fitness in non-agricultural environments. The BfN suggests providing phenotypic data including endpoints of ecological relevance (e.g. frost tolerance, seed dormancy, occurrence of volunteers) and a treatment group without agrochemicals. Data on volunteers may be a feasible first step to provide ecologically relevant information as volunteers may act as a genetic bridge to confer new traits from GM-maize to teosinte. Maize volunteers can be found in the EU on a regular basis as has been reported from Palaudelmàs et al. (2009) in Spain or from Pascher (2016) in Austria. The evidences from these	For event MON 87460 a comparative analysis was specifically conducted under drought conditions (EFSA Panel, 2012). Considering that there is no indication of an interaction between the events (see section 3.4.1.4 of the Scientific Opinion), it was not necessary to request the inclusion of field trials under drought conditions for the six-event stack maize The endpoints evaluated by the applicant to assess the agronomic and phenotypic characteristics of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 were in line with the scope of application EFSA-GMO-NL-2017-139 (no cultivation) and with the applicable EFSA guidelines. The GMO Panel considered it very unlikely that maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 will differ from conventional maize hybrid varieties in its ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 grains (further details are provided in Section 3.4.4.1 of the EFSA scientific opinion).

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				publications indicate that some maize varieties can tolerate frost and produce F1 progeny with viable seed. Maize therefore has the potential to survive as a volunteer or feral plant in Europe including regions with cold winters (Pascher 2016). The problem of volunteer maize is also addressed in other EU risk assessments such as the recommendation of the European Commission to remove volunteer maize plants in order to control Diabrotica spp. in the EU. References: Commission Recommendation 2014/63/EU of 6 February 2014 on measures to control Diabrotica virgifera virgifera Le Conte in Union areas where its presence is confirmed (OJ L 38/43 07.02.2014). Palaudelmàs, M., Peñas, G., Melé, E., Serra, J., Salvia, J., Pla, M., Nadal, A. and J. Messeguer (2009). Effect of volunteers on maize gene flow. Transgenic Res 18: 583–594. DOI 10.1007/s11248-009-9250-7. Pascher, K. (2016) Spread of volunteer and feral maize plants in Central Europe: recent data from Austria. Environ Sci Eur 28:30; DOI 10.1186/s12302-016-0098-1.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	Comment 6/ Federal Agency for Nature Conservation (BfN)	Teosinte has been reported to occur in Spain and France (EFSA 2016). As GM maize is mainly imported to Spain gene flow from GM maize to teosinte and vice versa must be considered in the risk assessment and monitoring. 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. (2012) 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 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. For applications with scope of import of maize seed information the occurrence of teosinte and GM-maize need to be collected in the PMEM. 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.	The GMO Panel took note of the comments raised by Germany. The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient (see Section 3.4.4.1 of the scientific opinion). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM maize plants resulting from grain spillage, and weedy or cultivated *Zea* plants is considered extremely low (EFSA, 2016). Even if cross-pollination would occur, the GMO Panel is of the opinion that environmental effects as a consequence of the spread of genes from occasional feral GM maize plants in Europe will not differ from that of conventional maize varieties.
Germany	BfN	II.5.3.4 Interactions of the GM plant with non-target organisms (NTOs)	Comment 7/ Federal Agency for Nature Conservation (BfN)	Import and processing of Bt maize are usually considered to have less environmental impact than cultivation. However, as the BfN pointed out in other Bt maize applications, exposure of the environment to Bt toxins should be considered in the ERA. For Bt proteins, in principle, the exposure route from feed, via manure into the environment has been demonstrated for cattle (Chowdhury et al. 2003a; Gruber et al. 2011; Gürtler et al. 2010, Paul et al. 2010) or pigs (Chowdhury et al. 2003b; Campos et al. 2018). To our understanding present studies are not sufficient to conclude that exposure of the environment	Considering the scope of application EFSA-GMO-NL-2017-139, which excludes cultivation, the environmental risk assessment of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 grains during transportation and processing.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				and thus effects on non-target organisms will be negligible. Instead, experimental evidence from the few studies available, demonstrates that Bt toxins will be present in feces if livestock is being fed with Bt crops. Consequently, for any market application of Bt crops, experiments should be presented in order to conclude on subsequent effects and risks for non-target organisms. Test protocols for both dung beetles and dung flies have been developed at the OECD level and may be adaptable to GMO. References: Campos, R.C., Holderbaum, D.F., Nodari, R.O., Hernandez, M.I.M., (2018) Indirect exposure to Bt maize through pig faeces causes behavioural changes in dung beetles. J. Appl. Entomol.,vol. 57, 117. Chowdhury, E.H., Shimada, N., Murata, H., Mikami, O., Sultana, P., Miyazaki, S., Yoshioka, M., Yamanaka, N., Hirai, N., Nakajima, Y. (2003) Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. Vet Hum Toxicol,vol. 45, 72–75. Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K.S., Saito, M., Nakajima, Y. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. Journal of Animal Science,vol. 81, 2546–2551. Gruber,H., Paul,V., Guertler,P., Spiekers, H., Tichopad, A., Meyer, H. H. D. & Müller, M. (2011) Fate of Cry1Ab Protein in Agricultural Systems under Slurry Management of Cows Fed Genetically Modified Maize (Zea mays L.) MON810: A Quantitative Assessment. Journal of Agricultural & Food Chemistry 59 (13), 7135–7144. Gürtler, S.P., Paul, V., Steinke, K., Wiedemann, S., Preißinger, W., Albrecht, C., Spiekers, H., Schwarz, F. J. & Meyer, H. H. D. (2010) Long-term feeding of genetically modified corn (MON810) - Fate of cry1Ab DNA and recombinant protein during the metabolism of the dairy cow. Livestock Science 131, 250-259. Paul,V., Guertler,P., Wiedemann,S., and Meyer.H.H. (2010). Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Res. 19: 4.	Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 seeds is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the six-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins will not alter this conclusion.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.6 Post-Market Environmental Monitoring Plan (PMEM)	Comment 8/ Federal Agency for Nature Conservation (BfN)	The scope of this application is for import, processing, and all uses for food and feed. The applicant provides an environmental monitoring plan, which remains very general. The monitoring plan has to be elaborated in more detail in order to meet the following requirements: • Provision of a fully specified list of monitoring parameters. • Application of standardised sampling methodologies: A basic prerequisite for comparing GMO monitoring data is the use of appropriate standard detection or analytical methods. Several standards specific for GMO monitoring are provided by the Association of German Engineers (VDI). They are available under http://www.vdi.eu/engineering/vdi-standards/. • Elaboration of a sampling concept. • In case of monitoring data being collected by external persons or institutions other than the applicant, binding agreements/contracts with third parties are requested which clearly determine what data are provided and how these data are made available. • Elaboration of the methods of data analysis including the statistical methods. • Application of the concept of adverse effects and environmental damages: Adverse environmental effects can only be determined if they are related to certain relevant subjects of protection (Bartz et al. 2009). The subject of protection is damaged if it is significantly adversely affected. The identification of a significant adverse effect should consider both its intensity (e.g. extent of loss) and the value of the impaired subject of protection (e.g. high value of protected species). The monitoring should be run in regions, where viable plant material of the GMO will be transported, stored, packaged, processed or used for food/feed. In case of substantial losses and spread of the GMO all receiving environments need to be monitored. Since traders may commingle the GMO with other commercial GM maize imported, processed or used for food/feed, the applicant is requested to explain how the monitoring will be designed to distinguish between potential adverse effects caused by the GMO and those caused by other GM maize. The Federal Agency for Nature Conservation is of the opinion that a detailed monitoring plan has to be provided before consent may be given. References:	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Bartz, R., Heink, U. & Kowarik, I. (2009): Proposed Definition of Environmental Damage Illustrated by the Cases of Genetically Modified Crops and Invasive Species. Conservation Biology 24 (3): 675–681.	
Germany	BfN	II.6.1 Interplay between Environmental Risk Assessment, Risk Management and PMEM	Comment 9/ Federal Agency for Nature Conservation (BfN)	The information necessary to conclude on the ERA is partly missing. Thus, the safety of the GMO cannot be fully assessed. Depending on those results the conclusions concerning case-specific monitoring may need to be revised.	In its risk assessment the GMO Panel did not identify potential adverse environmental effects from the maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. Therefore, case-specific for the GM maize is not required.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.6.2 Case Specific Monitoring (strategy, method and analysis)	Comment 10/ Federal Agency for Nature Conservation (BfN)	BfN does not share the opinion of the applicant that a case-specific monitoring is not necessary. Case-specific monitoring should be focused on pathways, where viable plant material of the GMO enters the environment. The applicant is requested to provide an appropriate case-specific monitoring plan comprising at least the following elements: i.) spillage or loss of the GMO during transport, storage, packaging, processing and use (feed and food), ii.) potential spread and persistence of the GMO within all environments, where substantial amounts of viable seeds are spilled, iii.) occurrence of teosinte in regions affected by transport, storage, packaging, processing, use and subsequently potential outcrossing of the transgenes, iv.) environmental fate of the Bt proteins resulting from sewage water, waste material, manure or by-products which may occur during processing, or use of non-viable plant material of the GMO as food/feed. For parameters i.) to iii.), the use of the following methods is recommended (http://www.vdi.eu/-engineering/vdi-standards/): - VDI-Guideline 4330 Part 10 "Floristic mapping of genetically modified plants their crossing partners and their hybrid offspring", - VDI-Guideline 4330 Part 5 "Guideline for the collection and preparation of plant samples for molecular biological analysis". If spread, persistence or accumulation of products of the GMO in the receiving environment occur, further observations of possible impacts on organisms, food chains and habitats in the specific environment are required. If risk management measures are envisaged, e.g. to minimize incidental spillage during transport, storage, packaging, processing or feed and food use, their efficacy should be monitored during case-specific monitoring (EFSA 2011). References: EFSA (2011). Scientific opinion. Guidance on the Post-Market Environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 9(8): 2316, 40 pp. 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.	As the ERA did not identify potential adverse environmental effects from the maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, no case-specific monitoring is required.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.6.3 General Surveillance (strategy, method)	Comment 11/ Federal Agency for Nature Conservation (BfN)	The applicant states that the general surveillance will be based on information gathered from the existing networks of COCERAL, UNISTOCK and FEDIOL. Data shall be collected by operators handling and using viable plant material of the GMO and reported to the authorisation holder, represented by EuropaBio. It remains unclear, how the authorisation holder/EuropaBio will inform operators about their surveillance function and how it will be assured that operators in duty for general surveillance show the necessary skills to detect environmental impacts of the GMO. Therefore, the applicant is requested - to name the national and local organisations and factories involved in the monitoring, - to prove that a sufficient number of local operators agree to contribute to the general surveillance, to provide a schedule with all relevant observation objects to be monitored, - to explain how local operators will be instructed and trained for conducting the general surveillance, to verify the necessary skills and expertise of local operators to detect adverse environmental impacts. In case the suggested operators are not capable to cover all relevant observation objects, further monitoring systems have to be established. The applicant does not suggest operators further down the food chain to be involved in the process of monitoring. The BfN does not approve this, because processed material may also be a cause of adverse effects. Therefore, the applicant is requested to involve also operators further down the food chain in the process of monitoring. The general surveillance plan has to focus on possible pathways how the GMO can get into the broader environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal and use of the GMO in environmental media. Beside the implementation of management and safety standards, the applicant is requested to provide an appropriate general surveillance plan comprising at least the above mentioned monitoring elements. The GMO may enter the environment together with other approved GM maize lines. Therefore, a special focus should be on possible combined effects.	Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BfN	II.6.4 Reporting the results of PMEM	Comment 12/ Federal Agency for Nature Conservation (BfN)	The applicant is required to report on the results of the monitoring including all issues of case-specific monitoring and general surveillance on an annual basis. Raw data have to be made available. The monitoring report should also deliver detailed information on i) actual volumes of the GMO imported into the EU, ii) the ports and silos where shipments of the GMO maize were unloaded, iii) the processing plants and users where viable plant material of the GMO was transferred to, iv) the amount of the GMO used on farms for feed, and v) transport routes of the GMO maize.	Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.1 Hazard identification and characterisation	Comment 1/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	The scope of application EFSA-GMO- NL-2017-139 covers import and processing of maize MON87427×MON87460×MON89034×1507×MON87411×59122 and all possible sub-combinations including all feed and food products containing, consisting of, or produced from the genetically modified maize MON87427×MON87460×MON89034×1507×MON87411×59122 and all possible sub-combinations. Cultivation is not covered by this application. All six underlying single events have already been risk assessed by EFSA. The five single events MON87427, MON87460, MON89034, 1507 and 59122 were approved for import and processing in the EU. For MON87411 the EFSA opinion was published on 28 June 2018. In line with the EFSA Guidance (EFSA, 2011) for the evaluation of maize MON87427×MON87460×MON89034×1507×MON87411×59122 the applicant refers to data given in the respective applications for authorisation of the single events MON87427 (EFSA-GMO-BE-2012-110), MON87460 (EFSA-GMO-NL-2009-70), MON89034 (EFSA GMO-NL-2007-37), 1507 (EFSA-GMO-RX-1507), MON87411 (EFSA-GMO-NL-2015-124) and 59122 (EFSA-GMO-NL-2005-12), respectively. We refer to the German comments which we have already submitted in conjunction with the risk assessment of these applications. Taken as a whole, the Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion that the entirety of available data supports the conclusion that maize MON87427×MON87460×MON89034×1507×MON87411×59122 is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended use. The same applies for all possible sub-combinations that can occur by natural segregation. Nevertheless, completion and/or clarification on some points of the dossier are recommended (see specific comments). In addition, the provided monitoring plan is incomplete at this stage and needs further elaboration for implementation.	The GMO Panel thanks Germany for the assessment.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.1.2.2 Information relating to the genetically modified plant	Comment 2/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	Overall, the presented data do not provide indications of interactions between the events in maize MON87427×MON87460×MON89034×1507×MON87411×59122 that may raise safety concerns. Potential risk associated with horizontal gene transfer The probability of a horizontal gene transfer via homologous recombination were shown to be very low and pose a negligible risk potential. The bioinformatics analyses for MON87427, MON89034, MON87411 and 59122 were performed with outdated databases (versions of 2016). However, more recent bioinformatics analyses from the application EFSA-GMO-NL-2017-144 were available for the evaluation. Moreover, it should be noted that the bioinformatics analyses of all individual events should ideally be carried out using identical versions of the same databases.	The information on the methodology and databases used to perform the bioinformatic analyses for all the events in the six-event stack maize have been assessed by the GMO Panel and considered to be appropriate. In particular, on the 18/3/2020 the bioinformatic analyses for the assessment of homologous recombination potential for events MON 87427, MON 89034, MON 87411, 1507 and 59122 have been submitted while for event MON87460 has been submitted on the 29/5/2020.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.1.3.4 Comparative analysis of composition	Comment 3/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	Production of material for compositional analysis was conducted at eight field sites in the U.S.A. during the 2014 field season. The locations are representative of the main maize cultivation region of the U.S.A. and reflect the conditions under which imported crops will be cultivated. At each location, maize MON87427×MON87460×MON89034×1507×MON87411×59122 treated and untreated with trait-specific herbicides glyphosate and glufosinate, the conventional counterpart and at least four non-GM maize reference varieties (18 non-GM varieties in total) were planted in a randomized complete block design with four replications. The statistical analysis of the compositional data assessed the differences between maize MON87427×MON87460×MON89034×1507×MON87411×59122 (treated and untreated) and the conventional counterpart as well as the equivalence of maize MON87427×MON87460×MON89034×1507×MON87411×59122 to the commercial reference hybrids according to EFSA guidelines. Overall, the performance and results of the compositional analysis do not give cause for concern. However, recent findings suggest that phosphinothricin acetyltransferase (PAT), known for its high affinity towards the active herbicidal ingredient glufosinate-ammonium (phosphinothricin), also shows N-acetyltransferase activities towards the amino acids tryptophan and aminoadipate (Christ et al. 2017). The study reports an ectopic accumulation of acetyl-aminoadipate and acetyl-tryptophan in multiple tissues of different GMO expressing PAT (soybean, canola, mustard and wheat). While acetyl-tryptophan is a naturally occurring metabolite found in numerous plant species, acetyl-aminoadipate has not yet been reported as an endogenous plant metabolite. The applicant should address a possible relevance of these findings for the food and feed safety of maize MON87427×MON87460×MON89034×1507×MON87411×59122. Christ B, Hochstrasser R, Guyer L, Francisco R, Aubry S, Hörtensteiner S, Weng J-K (2017) Non-specific activities of the major herbicide-resistance gene BAR. Nature Plants 3(12):937-945.	The GMO Panel took note of the comment and highlights that maize MON87427×MON87460×MON89034×1507×MON87411×59122 was not matter of assessment in Christ et al, 2017. Further considerations on Christ et al 2017 can be found at the following link: http://registerofquestions.efsa.europa.eu/roqFrontend/mandateLoader?mandate=M-2018-0043

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.1.4.1 Testing of newly expressed proteins	Comment 4/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	According to EFSA Guidance (2011) the applicant provided bioinformatics analyses results comparing the amino acid sequences of the newly expressed proteins to sequences of known proteins. The analyses were performed using different NCBI data bases in a version of 2016. While no relevant similarities to sequences of any known toxins were identified we wish to mention that meanwhile newer versions of these databases are available. Additionally, the same database versions and search strategies should be used for all analyses.	The applicant submitted voluntarily an update on bioinformatics analyses on the 16/03/2020. The EFSA GMO panel requested bioinformatics analyses using up-to-date databases for event MON 87460 at its clock 11 which was provided by the applicant on the 29/05/2020.
Germany	BVL, German CA	II.1.5.1 Assessment of allergenicity of the newly expressed protein	Comment 5/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	The potential allergenicity of the recombinant proteins CP4 EPSPS, CSPB, NptII, Cry1A105, Cry1Ab2, Cry1F, PAT, Cry3Bb1 and Cry34Ab1 has already been evaluated by EFSA in the context of previous applications. The bioinformatics analyses provided by the applicant for the current application were performed using a 2016 version of the FARRP database. While the results did not give any indication that may require a revision of the previous evaluation, the applicant should provide updated bioinformatics analyses regarding the allergenicity of the recombinant proteins using an updated version of the database.	The GMO Panel takes note of the comment. An up-to-date bioinformatics analyses was provided by the applicant.
Germany	BVL, German CA	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	Comment 6/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	The import documents should indicate that maize MON87427×MON87460×MON89034×1507×MON87411×59122 has not been approved for cultivation by the EC. In addition to the intended GM labelling a clear labelling, of maize MON87427×MON87460×MON89034×1507×MON87411×59122 indicating the tolerance to glyphosate and glufosinate-ammonium is recommended. Furthermore, appropriate measures have to be taken during transport, storage, and processing to avoid unintended release of germinable maize kernels into the environment. In this context, the applicant should inform all parties involved in the handling and processing maize MON87427×MON87460×MON89034×1507×MON87411×59122 about avoidance and control of spillage.	Labelling and monitoring is related to risk management; a final adoption of the PMEM plan falls outside the mandate of EFSA.
Germany	BVL, German CA	II.6 Post-Market Environmental Monitoring Plan (PMEM)	Comment 7/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	The monitoring plan is acceptable, but needs further elaboration for implementation. Therefore, the applicant is recommended to revise the monitoring plan during the initial implementation phase (after consent is given) and present this revised monitoring plan together with a first report one year after consent is given to be reassessed.	Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.6.2 Case Specific Monitoring (strategy, method and analysis)	Comment 8/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	According to the risk assessment, no adverse effects on the environment or human health were identified or were expected. Therefore, there is no necessity for a case-specific monitoring.	The GMO Panel took note of the comment.
Germany	BVL, German CA	II.6.3 General Surveillance (strategy, method)	Comment 9/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	The monitoring plan does not relate the monitoring activities to relevant protection goals. Even more, it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter, because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate information about the use of the product in food and feed to 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. In general, other sources of information e.g. peer-reviewed publications or on going research should be taken into account. However, the applicant should describe in detail how he would consider this information within General Surveillance.	Monitoring is related to risk management and not in the remit of the GMO Panel.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Germany	BVL, German CA	II.6.4 Reporting the results of PMEM	Comment 10/ Federal Office for Consumer Protection and Food Safety (BVL, German CA)	A report on GS activities on an annual basis is sufficient. Reporting should refer to the format introduced by the Commission Decision 2009/770/EC. The applicant is requested to state how the monitoring results will be published.	Monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.
Hungary	Ministry of Agriculture	II.1.1 Information relating to the recipient or (where appropriate) parental plants	HU2	Maize has a history of safe use, but GM maize generally, and the MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 stack in particular, has not. GM maize is cultivated for less than 20 years, and this maize only for a short time.	The EFSA GMO Panel thanks Hungary for the comment.
Hungary	Ministry of Agriculture	II.1.1 Information relating to the recipient or (where appropriate) parental plants	HU3	GM maize has the ability to cross-fertilize non-GM maize varieties and also teosinte, but non-GM maize does not harm the GM varieties. GM plants might now be considered as invasive species [Paull, J. (2018): Genetically Modified Organisms (GMOs) as Invasive Species Geography and Spatial Sciences, Journal of Environment Protection and Sustainable Development, Vol. 4, No. 3. http://orgprints.org/33327/1/Paull2018GMInvasiveSpeciesJEPSD.pdf]. If intraspecific hybridization were to happen asymmetrically, this does not exclude GM maize cross-pollinating teosinte and non-GM maize hybrids. The distance to produce hybrid maize safely is a minimum of 1000 meters between the site of seed production and another maize field, according to text books. The effects of the MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 stack on small mammals living on the fields were not considered by the applicants.	The GMO Panel took note of the comment.
Hungary	Ministry of Agriculture	II.1.2.1 Information relating to the genetic modification	HU4	(b) There is no history of safe use of any synthetic transgene, nor of GM maize. There are several cry toxins in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. They are toxic to mammals since they are able to bind to mammalian cells: M.A.A. Ibrahim, M. A. A. & E.F. Okasha, E. F. (2016): Effect of genetically modified corn on the jejunal mucosa of adult male albino rats. Experimental and Toxicologic Pathology 68, http://www.sciencedirect.com/science/article/pii/S0940299316	The GMO Panel took note of the comments by Hungary. Papers quoted by Hungary have been assessed by EFSA (e.g. EFSA, 2018; 2018a)

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				302056; Tayabali, A. F. & Seligy, V. L. (2000): Human cell exposure assays of Bacillus thuringiensis commercial insecticides: production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environmental Health Perspectives 108; Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. & De la Riva, G.A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271; Griffitts, J.S., Haslam, S.M., Yang, T., Garczynski, S.F., Mulloy, B., Morris, H., Cremer, P.S., Dell, A., Adang, M.J. & Aroian, R.V. (2005): Glycolipids as receptors for Bacillus thuringiensis crystal toxin. Science 307; Pusztai, A. & Bardocz, S. (2006): GMO in animal nutrition: potential benefits and risks. In: "Biology of Nutrition in Growing Animals" (ed. Mosenthin, R., Zentek, J. & Zebrowska, T.), Elsevier Limited, 513-540. p.; Rubio-Infante, N. & Monero-Fierros, L. (2015): An overview of the safety and biological effects of Bacillus thuringiensis cry toxins in mammals. Journal of Applied Toxicology 36. https://doi.org/10.1002/jat.3252. The cry toxins are immunogenic, allergic and might also act as adjuvants [Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467]. It is very likely that there is an interaction between the cry proteins produced in the transgenic maize. This interaction can be studied experimentally. Such interactions were observed by Bohn et al. [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722] and others. The safety of dsRNA is still questionable. No artificial dsRNA has a history of safe consumption. Not all nucleic acids break down in the small intestine, not even in the large bowel.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				(c) The fact that cry toxins are toxins and biologically active lectins was not considered by the applicant. Cry toxin can bind to mammalian cells and to gut. M.A.A. Ibrahim, M. A. A. & E.F. Okasha, E. F. (2016): Effect of genetically modified corn on the jejunal mucosa of adult male albino rats. Experimental and Toxicologic Pathology 68, http://www.sciencedirect.com/science/article/pii/S0940299316302056; Tayabali, A. F. & Seligy, V. L. (2000): Human cell exposure assays of Bacillus thuringiensis commercial insecticides: production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environmental Health Perspectives 108; Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. & De la Riva, G.A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271; Griffitts, J.S., Haslam, S.M., Yang, T., Garczynski, S.F., Mulloy, B., Morris, H., Cremer, P.S., Dell, A., Adang, M.J. & Aroian, R.V. (2005): Glycolipids as receptors for Bacillus thuringiensis crystal toxin. Science 307; Rubio-Infante, N. & Monero-Fierros, L. (2015): An overview of the safety and biological effects of Bacillus thuringiensis cry toxins in mammals. Journal of Applied Toxicology 36. https://doi.org/10.1002/jat.3252. Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467 (e) The donor organisms have no history of safe use, since they have never been used as food or feed.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.2.2 Information relating to the genetically modified plant	HU5	MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize contains multiple copies of the herbicide resistance genes to tolerate being sprayed with glyphosate and glufosinate. The interaction between those multiple gene copies cannot be excluded on the basis of evidence provided, and are probable, since the amounts of the transgenic proteins expressed by the individual events and in the stack (1.2.2.1) do not match in all cases (see Tables 1-11); In this stack 1) there is an ARM gene, the NptII, the use of which should be discouraged in the EU; 2) there are six synthetic cry genes coding for the transgenic proteins Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1. Their genes might not interact, but the transgenic proteins might have an additive or even synergistic effect with each other on a cellular/metabolic level, and not only on the target organisms, but also on other species consuming them [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): *Daphnia magna* negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722]; 3) The safety of the DvSnf7 dsRNA, which in theory helps maize to tolerate draught better, is still questionable. riRNA might have off-target effects [Latham, J.R. & Wilson AK (2015): Off-target Effects of Plant Transgenic RNAi: Three Mechanisms Lead to Distinct Toxicological and Environmental Hazards. Presented at GMO-Free Regions, BERLIN]. There are examples to show that utilizing RNA interference (RNAi) in feed has an effect on the gene expression of the hosts. There is also evidence that plant genes influence the metabolism of the consuming organisms, although they do not become integrated into their genomes. Pastrello, C., Tsay, M., McQuaid, R., Abovsky, M., Pasini, E., Shirdel, E., Angeli, M., Tokar, T., Jamnik, J., Kotlyar, M., Jurisicova, A., Kotsopoulos, J., El-Sohemy, A. & Jurisica, I.	The molecular characterisation data establish that the events stacked in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 have retained their integrity. Protein expression analyses showed that the levels of the newly expressed proteins are similar in the six-event stack maize and in the single events, except for the expected difference for the CP4 EPSPS and PAT protein levels resulting from the combination of the MON 87427 and MON 87411 events, and 1507 and 59122 events in the six-event stack respectively. No indications of interactions that may affect the integrity of the events and the levels of the newly expressed proteins in this four-event stack maize were identified. 1) The GMO Panel took note of this comment. The applicant in accordance with Article 4(2) of Directive 2001/18/EC and with Reg (EU) 503/2013, should aim to develop GMOs without the use of antibiotic resistance marker genes. 2) Given that environmental exposure of non-target organisms to spilled maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 grains or occasional feral GM maize plants arising from spilled six-event stack maize grains is limited, and because ingested dsRNA and proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the six-event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry proteins (as mentioned in Section 3.4.1.4 of the EFSA GMO Panel Scientific Opinion on application EFSA-GMO-NL-2017-139) would not alter this conclusion. 3) DvSnf7 dsRNA helps in conferring resistance to corn rootworms (*Diabrotica* spp.) The assessment of DsRNA and derived siRNAs with regards to food and feed safety is addressed in Section 3.4.3.3 of the Scientific Opinion. An overview of EFSA's activities on the risk assessment of RNAi-based GMPs is given in Papadopoulou et al. (2020)

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				(2016): Circulating plant miRNAs can regulate human gene expression in vitro. Scientific Reports 6, doi:10.1038/srep32773; Kamath, R. S., Martinez-Campos, M., Zipperlen, P., Fraser, A. G. & Ahringer, J. (2001): Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biology 2, doi: 10.1186/gb-2000-2-1-research0002; 4) The proteins CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1 proteins expressed in this stack have not been properly tested for their safety. 1.2.2.3 Although there might not be an interaction between the inserts the transgenes express resistance to, there is a likely interaction between the herbicide residues and metabolites. Those interactions cannot be predicted but can be tested for and those tests have not been carried out. 1.2.2.4 Recombination between the inserts is unlikely to occur in this stack, although interactions between the transgenic insecticidal proteins are highly likely [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722]. 1.2.2.5 The possibility of HGT occurring has been underestimated. There is evidence of HGT with the spread of herbicide/antibiotic resistance and the presence of NptII can aid the process. In addition, HGT might provide selective advantages in an environment where glyphosate and gfufosinate are present at low concentrations. HGT estimation did not consider the fact that the transgenes in GM plants are usually synthetic versions of the genes occurring in nature; they are expressed in a matrix different than their original one(s); the transgene(s) are under the influence of different regulatory elements to help maximize protein expression. Under these conditions HGT might occur with higher frequency, especially in the gut microbiome. Transgenes originating from GM plants were detected in several animal organs and their detectability depends on the sensitivity of the methods used: Tudisco, R., Mastellone, V., Cutrignelli, M. I., Lombardi, P.,	4) The microbial DNA similarity search, conducted for the assessment of potential homologous recombination between plants and microorganisms in the environment, via horizontal gene transfer has been assessment according to the Explanatory Note (EFSA 2017). The EFSA GMO Panel assessed in previous opinions the probability and potential adverse effects of HGT of the recombinant DNA for the single events (see Table 1 in the Scientific Opinion) including the case of MON 87460 (EFSA GMO Panel, 2012; 2019b). This assessment included consideration of homology-based recombination processes, as well as non-homologous end joining and microhomology-mediated end joining. Possible fitness advantages that the bacteria in the receiving environments would gain from acquiring recombinant DNA were considered. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified. The applicant submitted updated bioinformatic analysis for each of the single events in order to assess the possibility for HGT by HR. The GMO Panel concludes that the unlikely, but theoretically possible, horizontal transfer of recombinant genes from this six-event stack maize to bacteria does not raise any environmental safety concern.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Bovera, F., Mirabella, N., Piccolo, G., Calabrò, S., Avallone, L. & Infascelli, F. (2010): Fate of transgenic DNA and evaluation of metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal 4; Calabrò, S., Cutrignelli, M.I., Moniello, G., Grossi, M., Mastellone, V., Lombardi, P., Peroa, M.E. & Infascelli, F. (2015): Genetically modified soybean in a goat diet: Influence on kid performance. Small Ruminant Research 126; Grønsberg, I.M., Nordgård, L., Fenton, K., Hegge, B., Nielsen, K.M., Bardocz, S., Pusztai, A. & Traavik, T. (2011): Uptake and organ distribution of feed introduced plasmid DNA in growing or pregnant rats. Food Nutrition Science 2, doi:10.4236/fns.2011.24053. Exogenous DNA was detected in human blood [Spisak, S., Solymosi, N., Ittzés, P., Bodor, A., Kondor, D. et al. (2013): Complete Genes May Pass from Food to Human Blood. PLoS ONE 8, doi:10.1371/journal.pone.0069805]. Although transgenic (plant) genes do not recombine with the genetic material of human or animal cells, but they can influence their metabolism.	
Hungary	Ministry of Agriculture	II.1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects	HU6	The probability of transgenic DNA entering and recombining with the cells of the microbiome has been studied and proved possible. In an experiment the full transgenic plant DNA was detected after several passages in the microbes of ileostomy patients after consuming milkshake containing GM soybeans (Netherwood et al 2004). The reason for not being able to detect transgenic DNA in more studies is the low sensitivity of the detection methods used.	The GMO Panel took note of the comments. Genomic DNA can be a component of food/feed products derived from maize. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and domesticated animals, and in other environments may be exposed to fragments of DNA, including the recombinant fraction of such DNA. Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally-located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	HU7	Statistical analysis should have been performed with the GM maize and its isogenic comparator grown at the same location at the same time. Using one commercial variety is sufficient to account for natural variability. No proper non-GM hybrid was produced when the stack MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize was bred, and therefore the MPA640B line was used as a near-isogenic control. In addition, 18 commercial maize lines were used "representing a range of genetic backgrounds and phenotypic characteristics" and the "full range of natural variability". According to Hungarian experts one commercial line should have been sufficient to account for natural variability. The use of 18 varieties serve only to widen the range and cover up compositional differences between the GM stack and its comparator.	The field trial design and the statistical analysis were in line with the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a).

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.3.4 Comparative analysis of composition	HU8	Statistically significant differences in forage composition were detected between the herbicides-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for calcium, moisture, phosphorus, ADF and NDF. Statistically significant differences in seed composition were found between the herbicides-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for alanine, aspartic acid, phenylalanine, serine, threonine, tyrosine, carbohydrates by calculation, glutamic acid, isoleucine, leucine, valine, protein, manganese, histidine and proline. Statistically significant differences in forage composition were found between the non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for histidine, proline, histidine, proline, ADF and NDF. Statistically significant differences in seed composition were found between the non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for: alanine, aspartic acid, glutamic acid, isoleucine, leucine, linolenic acid, arachidic acid, eicosenoic acid, total fat, vitamin A, vitamin B6, vitamin E, carbohydrates by calculation, total dietary fiber, calcium, copper, iron, magnesium, manganese, ferulic acid, raffinose, proline and protein. The statistically significant differences indicate that this GM maize stack and its control are different. The wide range of values found with the commercial varieties cannot show the absence of unintended changes if they had occurred. Based on the differences listed above Hungarian experts do not concur with the conclusion that MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize is compositionally similar to its conventional counterpart, and it can be a significant contributor to compositional variability in maize.	The GMO Panel assessed all significant differences between the six-event stack maize and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM maize reference varieties. None of the differences identified in forage and grain composition between the six event stack maize and the non-GM comparator needed further food/feed safety assessment except for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. The relevance of these changes was further discussed in Section 3.4.3.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	HU9	MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize or any other GM maize has no history of safe use. Statistically significant differences in phenotypic characteristics were found between the herbicide-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for early stand count (#/two rows), days to 50% pollen shed, Ear height (cm), final stand count (#/two rows), test weight (kg/hl), and days to 50% silking. Statistically significant differences in phenotypic characteristics were found between the non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for early stand count (#/two rows), days to 50% pollen shed, days to 50% silking, final stand count (#/two rows), test weight (kg/hL).	For the six-event stack maize (not treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early stand count, days to 50% pollen shed, days to 50% silking, root lodged plants, final stand count and test weight. All these endpoints fell under equivalence category I except for root lodged plants, for which the test of equivalence was not applied (because the variation between the non-GM commercial varieties was estimated to be 0). For the six-event stack maize (treated with the intended herbicides), statistically significant differences with the non-GM comparator were identified for early stand count, days to 50% pollen shed, days to 50% silking, ear height, root lodged plants, final stand count and test weight. All these endpoints fell under equivalence category I or II except for root lodged plants, for which the test of equivalence was not applied. The changes in root lodged plants were further assessed and found not to have a safety impact. The GMO Panel concluded that none of the other differences needed further assessment.
Hungary	Ministry of Agriculture	II.1.3.6 Effects of processing	HU10	There were statistically significant differences in composition of both herbicide(s)-treated and not treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator, therefore it should be considered different from conventional maize.	Maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 will undergo existing production processes used for conventional maize. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the six-event stack maize into food and feed products is not expected to result in products being different from those of conventional non-GM maize varieties.
Hungary	Ministry of Agriculture	II.1.3.7 Conclusion	HU11	There were statistically significant differences in composition of both herbicide(s)-treated and not treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator, therefore Hungarian experts disagree with the conclusion that no differences were identified in compositional data of forage and grain from MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122, or in its agronomic and phenotypic characteristics, that would require further assessment with regards to food and feed safety.	The GMO Panel concluded that none of the differences identified needed further assessment, except (for agronomic/phenotypic characteristics) for the changes in root lodged plants and (for composition) for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. These differences were further discussed in Section 3.4.4 and 3.4.3 and found not to have an impact on safety or nutrition.
Hungary	Ministry of Agriculture	II.1.4.1 Testing of newly expressed proteins	HU12	1.4.1: One of the newly expressed transgenes in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize codes for an ARM gene; there is also a novel dsRNA in this stack. In addition to proteins resulting in resistance to the herbicides glyphosate and glufosinate, there are 6 cry proteins in this stack, which are able to interact with each other on a cellular/metabolic level in the consumer species [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): Daphnia	The GMO Panel thanks Hungary for the comments. The assessment of the protein newly expressed in a stack is based on the assessment of these in the context of the respective single event plants, on updated bioinformatics, new additional information, if any, and on considerations on their possible interactions of relevance for food and feed safety. In general, the use of recombinant "surrogate" proteins for safety studies is in line with Reg (EU) 503/2013. The equivalence of the "surrogate" protein to the plant expressed protein is thoroughly evaluated, and differences, if any, are discussed with

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722]. The toxicity testing of the synthetic transgenic proteins CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins expressed in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 was performed only with the bacterial recombinant form of these proteins, and never with those isolated from the transgenic plants. While the bacterial recombinant cry proteins are not glycosylated, the plant-produced proteins might be [Latham, J. R., Love, M. & Hilbeck, A. (2017): The distinct properties of natural and GM cry insecticidal proteins, Biotechnology and Genetic Engineering Reviews 31, DOI: 10.1080/02648725.2017.1357295]. Cry proteins are able to bind to mammalian cells: M.A.A. Ibrahim, M. A. A. & E.F. Okasha, E. F. (2016): Effect of genetically modified corn on the jejunal mucosa of adult male albino rats. Experimental and Toxicologic Pathology 68, http://www.sciencedirect.com/science/article/pii/S0940299316302056; Tayabali, A. F. & Seligy, V. L. (2000): Human cell exposure assays of Bacillus thuringiensis commercial insecticides: production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environmental Health Perspectives 108; Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. & De la Riva, G.A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271; Griffitts, J.S., Haslam, S.M., Yang, T., Garczynski, S.F., Mulloy, B., Morris, H., Cremer, P.S., Dell, A., Adang, M.J. & Aroian, R.V. (2005): Glycolipids as receptors for Bacillus thuringiensis crystal toxin. Science 307; Pusztai, A. & Bardocz, S. (2006): GMO in animal nutrition: potential benefits and risks. In: "Biology of Nutrition in Growing Animals" (ed. Mosenthin, R., Zentek, J. & Zebrowska, T.), Elsevier Limited, 513-540. p.; Rubio-Infante, N. & Monero-Fierros, L. (2015): An overview of the safety and biological effects of Bacillus thuringiensis cry toxins in mammals. Journal of Applied Toxicology 36. https://doi.org/10.1002/jat.3252.	regards to their possible impact on the adequacy and representativeness of the test material used in safety studies. The GMO Panel agrees with Hungary on the technical challenges offered in the identification of recombinant Cry proteins equivalent to the plant ones; these have been taken into account in the context of the assessment of the respective single-event maize. Finally, papers quoted by Hungary have been assessed by EFSA (e.g. EFSA, 2018a)

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Cry toxins are immunogenic, allergic and might also act as adjuvants [Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467]; and are able to bind to gut cells of mammals [Zdziarski, I. M., Carman, J. A., Edwards, J. W. (2018): Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. Food and Nutrition Sciences 9, DOI:10.4236/fns.2018.96058]. The result of acute toxicity testing cannot indicate any chronic toxicity, especially in case of lectins, such are the cry toxins expressed in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. 1.4.1.3: The stability of the newly expressed proteins under relevant processing and storage conditions was determined using the bacterial recombinant version of the proteins. The effect of heat treatment might be different for the transgenic proteins from the GM plant in a protective matrix; degradation of the transgenic protein from the GM plant should have been determined experimentally. The in vitro digestibility studies have no relevance to digestibility in the bowel, especially in case of lectins. Cry- and cry-type proteins are known to survive passage through the intestines and degrade only partially to a functionally, biologically and immunologically still active core protein. Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami, O., Shimada, N., Guruge, K.S., Saito, M. & Nakajima, Y. (2003): Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. Journal of Animal Science 81; Deaville, E.R. & Maddison, B.C. (2005): Detection of Transgenic and Endogenous Plant DNA Fragments in the Blood, Tissues and Digesta of Broilers. Journal of Agricultural and Food Chemistry 53; Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C., Gelencsér, E., Jánosi, A. et al. (2011): Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. PLoS One, 6,	In relation to the assessment of Cry proteins for their allergic/adjuvant potential, the GMO Panel followed its guidance documents to assess the allergenic potential of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (EFSA GMO Panel, 2011; Regulation 503/2013). The conclusions of the assessment of allergenicity of the newly expressed proteins in the context of this application is described in section 3.4.3.4 of the Scientific Opinion. The GMO Panel considers that there are no indications that the newly expressed proteins in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 may be allergenic, in the context of this application. The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed. For additional information on adjuvanticity, please see additional references cited in the EFSA opinion referring to an EFSA document on adjuvanticity of Cry1Ac and an EFSA external report on adjuvanticity in general (EFSA, 2018b; Parenti et al., 2019). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this six-event stack maize might be adjuvants able to enhance an allergic reaction.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177; 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, doi: 10.3389/fenvs.2015.00071. 1.4.1.4: The resistance testing of the synthetic transgenic proteins CP4 EPSPS, cspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 present in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 to proteolytic enzymes in vitro was performed with the bacterial recombinant form of the proteins and never with those isolated from the transgenic plants. In vitro digestibility studies have no relevance to digestibility in the bowel, especially in case of lectins. Cry proteins are known to survive passage through the gastrointestinal tract in a biologically and immunologically intact form. 1.4.1.5: Neither the transgenic proteins CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1, nor their source organisms have a history of safe use, since they have never been consumed as food or feed. The transgenes are synthetic versions of the genes occurring in nature; they are expressed in a matrix different than their original one(s), and are under the influence of different regulatory elements aimed to maximal expression. Cry- and cry-type proteins might have an additive or synergistic effect on the host cells when consumed [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722] It is very detrimental that the toxin and allergen databases do not contain the sequences of the cry toxins themselves, because it makes the bioinformatic analysis unreliable while judging the safety of any transgenic proteins.	

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.4.2 Testing of new constituents other than proteins	HU13	1.4.2 and 1.4.3: Several compositional differences were found in seed and forage composition between the herbicide-treated and non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 and its comparator, see points 1.3.4 and 1.3.5. The weight of evidence guidelines to judge toxicity should have alerted the risk assessors to ask for further experiments, since 1) the transgenic proteins have no history of safe use; 2) several of the transgenic proteins show structural similarity to toxins, such as the cry toxins themselves; 3) when it comes to lectins, even a negligible amount of the proteins that are able to bind to cell surface receptors is enough to accumulate with time and exert biological/immunological/toxicological effects. The cry toxins are immunogenic, allergic and might also act as adjuvants [Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467], they exert biological/metabolic effects [Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. & De la Riva, G.A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271] and can bio-accumulate and exert toxic effects; 4) the in vitro digestibility of the transgenic proteins has no relevance to true digestibility in the gut, especially in case of lectins; 5) the deactivation of the transgenic proteins during processing has not been shown. Under these circumstances it would have been well justified to conduct 28-day oral toxicity studies with the combination of the transgenic proteins CP4 EPSPS, cspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1.	The GMO Panel took note of the comment. The assessment of the new proteins expressed in this stack maize is based on their assessment in the single event maize, on updated bioinformatics, new additional studies, and on the evaluation of potential interactions of relevance for food and feed safety, in line with Regulation (EU) 503/2013. Please see Section 3.4.3.3 of the Scientific Opinion for further information.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.4.2 Testing of new constituents other than proteins	HU15	1.4.2-1.4.4: There were several compositional differences found in seed and forage composition between the herbicide treated and non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator.	The outcome of the comparative assessment identified several compounds (ADF in forage, and protein, arginine, glycine, leucine, lysine and manganese in grain) needing food/feed safety assessment. As indicated in section 3.4.3.6 of the scientific opinion, the biological relevance of these compounds, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment. Based on the nutritional assessment, the GMO Panel concluded that the compositional changes observed in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 do not represent any concern from a nutritional point of view.
Hungary	Ministry of Agriculture	II.1.4.4 Testing of the whole genetically modified food or feed	HU14	1.4.4.1: There were several compositional differences found in seed and forage composition between the non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator. dsRNA might represent unique risks for the consumers: Pastrello, C., Tsay, M., McQuaid, R., Abovsky, M., Pasini, E., Shirdel, E., Angeli, M., Tokar, T., Jamnik, J., Kotlyar, M., Jurisicova, A., Kotsopoulos, J., El-Sohemy, A. & Jurisica, I. (2016): Circulating plant miRNAs can regulate human gene expression in vitro. Scientific Reports 6, doi:10.1038/srep32773; Kamath, R. S., Martinez-Campos, M., Zipperlen, P., Fraser, A. G. & Ahringer, J. (2001): Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. Genome Biology 2, doi: 10.1186/gb-2000-2-1-research0002. No artificial dsRNA has a history of safe consumption. Not all nucleic acids break down in the small intestine or even in the entire gastrointestinal tract. There is no history of safe use neither for the transgenic proteins CP4 EPSPS, cspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins or their source organisms, since they have never been consumed as food or feed. Because of the possible interaction between the transgenic insecticidal proteins, it would have been justifiable to conduct a 90-day feeding study with rodents. The result of acute toxicity testing cannot indicate chronic toxicity, especially in case of lectins. If a lectin protein does not exert any acute toxicity, it does not mean it has no chronic	The GMO Panel took note of the comment. The assessment of the dsRNA and it derived siRNAs with regards to food and feed safety is addressed in the Scientific Opinion (see Section 3.4.3.3). An overview of EFSA's activities on the risk assessment of RNAi-based GMPs is given in Papadopoulou et al. (2020) The GMO Panel took into account the assessment of the newly expressed protein conducted in the single event-maize, updated bioinformatics analysis and new additional studies, if any, and considered potential interactions of relevance for food and feed safety based on their biological functions. The GMO Panel did not consider necessary to conduct further studies on the new proteins, or their combinations.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				toxic effects, since the continuous intake of lectins over a longer time allows their binding to newly freed or newly formed receptors appearing on cell surfaces and by binding to those they can exert their chronic toxic or biological/physiological effects. When rats were fed a diet of GM maize modified with cry proteins for about 6 months, they showed changes in the lining of their stomachs; the normally tightly held "tight junction" had gaps in at least 30% of their tight junctions; in their stomach several of the pits were dilated and contained debris or mucus, while the controls were normal [Zdziarski, I. M., Carman, J. A., Edwards, J. W. (2018): Histopathological Investigation of the Stomach of Rats Fed a 60% Genetically Modified Corn Diet. Food and Nutrition Sciences 9, DOI:10.4236/fns.2018.96058]. Evidence exists that cry toxins are toxic also to human cells [Mizuki, E. et al., (1999): Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell- killing action on human cancer cells. Journal of Applied Microbiology 86]. 1.4.4.2: Since this crop is sprayed with a mixture of herbicides, their residues and metabolites are present on the seeds/forage. The evidence of glyphosate toxicity is accumulating (Woźniak, E., Sicińska, P., Michałowicz, J., Woźniak, K., Reszka, E., Huras, B., Zakrzewski, J. & Bukowska, B. (2018): The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessment. Food and Chemical Toxicology 120. https://www.sciencedirect.com/science/article/pii/S0278691518304800) and has an effect even on reproduction. The effects of the insecticidal (cry) transgenic proteins have not been studied on mammals. Without performing those experiments the combined effects of cry toxins are unknown. 1.4.4.4: Hungarian experts do not agree with the opinion expressed in the Application, that the safety of the CP4 EPSPS, cspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1 proteins and DvSnf7 dsRNA for humans and animals have been demonstrated. The data in part 1.3 demonstrated compositional differences between both in herbicide(s)-treated and non-treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator. In addition, there is the possibility of an interaction between the insecticidal transgenic proteins, especially in the presence of an untested combination of herbicide residues and	The risk assessment of herbicide residues in GM plants is not in the remit of the GMO Panel, but is performed by EFSA's Pesticides Unit. Animal studies on food/feed derived from the six event stack maize are considered not necessary by the GMO panel: based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety related to the stability and expression of the inserts or to interactions between the transformation events, and no modifications of toxicological concern in the composition of the six-stack maize have been identified. This is in line with Regulation (EU) 503/2013.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				metabolites. Therefore a 90-day rodent study should have been performed to assess the nutritional/toxicological safety of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. The toxicological assessment is not convincing and cannot guarantee the safety of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize or show that it is as safe as any non-GM maize variety and will not have any adverse effect on the health of consumers.	
Hungary	Ministry of Agriculture	II.1.5.1 Assessment of allergenicity of the newly expressed protein	HU16	1.5.1: The cry toxins are lectins and are known to be allergenic: Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467; Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. & De la Riva, G.A. (2000): Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271. The MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize crop is sprayed with an untested herbicide mixture and the residues and metabolites of those might increase the risk of allergic reactions. 1.5.1.1: Neither the transgenes, nor their source organisms have a history of safe use, since none was consumed as food or feed earlier. In addition, the crop contains dsRNA, representing unpredictable hazards with unknown consequences. MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize is sprayed with an untested herbicide residue- and metabolite mixture in combination. 1.5.1.2.: Pepsin resistance and in vitro digestibility tests in vitro has no relevance to transgenic protein degradation in the gut, especially when several of the transgenic proteins are lectins, resisting – at least partially – protein degradation in vivo [Walsh, M.C., Buzoianu, S.G., Gardiner, G.E., Rea, M.C.,	In relation to the assessment of Cry proteins and their allergic/adjuvant potential, the GMO Panel followed its guidance documents to assess the allergenic potential of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (EFSA GMO Panel, 2011; Regulation 503/2013). The conclusions of the assessment of allergenicity of the newly expressed proteins in the context of this application is described in section 3.4.3.4 of the Scientific Opinion. The GMO Panel considers that there are no indications that the newly expressed proteins in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 may be allergenic, in the context of this application. The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed. For additional information on adjuvanticity, please see additional references cited in the EFSA opinion referring to an EFSA document on adjuvanticity of Cry1Ac and an EFSA external report on adjuvanticity in general (EFSA, 2018b; Parenti et al., 2019). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this six-event stack maize might be adjuvants able to enhance an allergic reaction. In relation to the comment on *in vitro* digestibility tests, the studies were considered as additional information for the safety assessment of the newly expressed proteins. The EFSA GMO Panel published a guidance document on allergenicity providing additional considerations on the *in vitro* protein degradation studies in 2017. In Annex B of this document, the EFSA GMO Panel proposes a refined *in vitro* digestion test that extends the conditions currently used in the classical pepsin resistance test in order to better reflect the range of conditions found *in vivo*. The test proposed includes additional conditions more representative of the gastric environment with regard to pH and pepsin levels, together with an intestinal digestion phase. In addition, more informative read-outs of the test are laid out which define the

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				Gelencsér, E., Jánosi, A. et al. (2011): Fate of transgenic DNA from orally administered Bt MON810 maize and effects on immune response and growth in pigs. PLoS One, 6, http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0027177]. 1.5.1.4: The facts that 1) cry proteins are immunogenic and allergenic, 2) it has not been proven that the transgenic proteins degrade in the gut, 3) in immunology there is no correlation between the effect and immunogenic quantity, and even a small amount of protein is able to invoke an anaphylactic shock, were not taken into account by the applicant.	extent to which either the intact protein or resistant fragments remain after *in vitro* digestion. However, the EFSA GMO Panel considered that additional investigation is needed before any additional recommendation in the form of guidance for applicants can be provided on the proposed *in vitro* protein digestibility tests. To this end, an interim phase period, which is currently ongoing, was considered necessary to evaluate the proposed revisions to the *in vitro* gastrointestinal digestion test. The outcome of the EFSA procurement providing experimental data on the testing of control proteins have recently been published in the EFSA website here: http://www.efsa.europa.eu/en/supporting/pub/en-1765. In a subsequent step, EFSA will assess whether the test adds value to the allergenicity risk assessment and, if so, what further actions are needed to move forward the field.
Hungary	Ministry of Agriculture	II.1.5.2 Assessment of allergenicity of the whole genetically modified plant	HU17	According to Hungarian experts neither the safety of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize, nor its similar allergic potential to non-GM maize was proven by the applicant.	The GMO Panel takes note of the comment.
Hungary	Ministry of Agriculture	II.1.5.3 Conclusion of the allergenicity assessment	HU18	The adjuvanticity of the combination of cry toxins in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize should have been studied, since cry toxins are lectins and structurally belong to the to A-B type toxin family, just like cholera toxin does. They are known to be able to act as adjuvants [Santos-Vigil, K. I., Ilhuicatzi-Alvarado, D., García-Hernández, A. L., Herrera-García, J. S., & Moreno-Fierros, L. (2018): Study of the allergenic potential of Bacillus thuringiensis Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy. International immunopharmacology 61, https://www.sciencedirect.com/science/article/pii/S1567576918302467].	In relation to the assessment of Cry proteins and their allergic/adjuvant potential, the GMO Panel followed its guidance documents to assess the allergenic potential of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 (EFSA GMO Panel, 2011; Regulation 503/2013). The conclusions of the assessment of allergenicity of the newly expressed proteins in the context of this application is described in section 3.4.3.4 of the Scientific Opinion. The GMO Panel considers that there are no indications that the newly expressed proteins in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 may be allergenic, in the context of this application. The GMO Panel has previously evaluated the safety of the newly expressed proteins, and no evidence of adjuvanticity were identified in the context of the applications assessed. For additional information on adjuvanticity, please see additional references cited in the EFSA opinion referring to an EFSA document on adjuvanticity of Cry1Ac and an EFSA external report on adjuvanticity in general (EFSA, 2018b; Parenti et al., 2019). The GMO Panel did not find indications that the Bt proteins at the levels expressed in this six-event stack maize might be adjuvants able to enhance an allergic reaction.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.1.6.1 Nutritional assessment of the genetically modified food	HU19	According to the opinion of Hungarian experts neither the safety, nor the similar nutritional value of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize to non-GM maize was proven by the applicant. There are six cry toxins in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize, with a possibility of interacting with each other on a cellular/metabolic level, when consumed, as it was shown earlier [Bøhn, T., Macagnan Rover, C. & Semenchuk. P. R. (2016): Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91, https://www.sciencedirect.com/science/article/pii/S0278691516300722]. The transgenic plant cry proteins might be glycosylated, increasing their allergic potential [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, doi: 10.3389/fenvs.2015.00071], while the bacterial recombinant versions used in all the tests are not. In addition, the transgenic proteins act in the presence of an untested mixture of herbicide residues and their metabolites. This should warrant a 90-day feeding study with laboratory rodents and possible with all other species exposed to feed made of herbicide-sprayed MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize.	The outcome of the comparative assessment identified several compounds (ADF in forage, and protein, arginine, glycine, leucine, lysine and manganese in grain) needing food/feed safety assessment. As indicated in section 3.4.3.6 of the scientific opinion, the biological relevance of these compounds, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes were considered during the nutritional assessment. Based on the nutritional assessment, the GMO PAnel concluded that the compositional changes observed in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 do not represent any concern from a nutritional point of view.
Hungary	Ministry of Agriculture	II.2 Exposure assessment — anticipated intake or extent of use	HU20	The dietary intake should have been calculated as the sum of all the cry proteins present in MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. A toddler is exposed to 0.3 mg/kg bw cry toxins daily, which is a high intake. In addition, lectins tend to bioaccumulate in the gut/body, which fact was not taken into account when calculating the margins of exposure. Neither MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize, nor its transgenic proteins and their donor organisms have a history of safe use, and the calculation of exposure to transgenic proteins does not consider the lectin-nature of 6 transgenic cry proteins.	As requested by IR 503/2013, human (chronic and acute) dietary exposure to the different newly expressed proteins (CP4 EPSPS, CspB, NPTII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1) was estimated considering a 100% replacement scenario that it is considered overly conservative when assessing the intake of these proteins. Additionally, potential losses of the newly expressed proteins during processing are not considered, which also implies an overestimation of the current dietary exposure

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.3 Risk characterisation	HU21	The risk characterisation section does not consider the fact that: a) the residues and metabolites of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize sprayed with an untested mixture of herbicideswhich remain in the seeds and forage and might endanger human and animal health [Woźniak, E., Sicińska, P., Michałowicz, J., Woźniak, K., Reszka, E., Huras, B., Zakrzewski, J. & Bukowska, B. (2018): The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessment. Food and Chemical Toxicology 120. https://www.sciencedirect.com/science/article/pii/S0278691518304800]; b) The combined effects of 6 synthetic/plant optimized cry proteins are unpredictable. It is likely that they interact with each other on a cellular and metabolic level; c) There are several statistically significant differences between MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator(s); d) The MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize contain an ARM gene, the NptII, the use of which should be discouraged in the EU; e) The criteria for characterising the transgenic proteins are based on misleading presumptions, such as that 1) the transgenic proteins degrade in vitro (lectins degrade only partially in vivo); 2) similarity to known toxins and allergens, while cry toxins themselves are toxins (Mizuki, E. et al., (1999): Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell- killing action on human cancer cells. Journal of Applied Microbiology 86) and allergens; 3) the quantity of the protein consumed is negligible or small (in allergy there is no relationship between effect and allergen quantity, and lectins also tend to bioaccumulate); 4) the bacterial recombinant version of the transgenic proteins were characterised instead of the proteins isolated from the GM plant; 5) the history of safe use which is not proven.	a) The risk assessment of herbicide residues in GM plants is not in the remit of the GMO Panel, but is performed by EFSA's Pesticides Unit. b) The GMO Panel considers that taken together, all the data in the dossier are sufficient to conclude on the absence of interactions between the events (including the newly expressed proteins) that would raise safety concerns in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. c) Differences observed in the between MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its non-GM comparator were taken into account considering the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference (equivalence test). The GMO Panel concludes that none of the differences identified needs further assessment, except for the changes in root lodged plants, in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. These differences are further discussed in Section 3.4.4 and 3.4.3. d) The GMO Panel took note of this comment. The applicant in accordance with Article 4(2) of Directive 2001/18/EC and with Reg (EU) 503/2013, should aim to develop GMOs without the use of antibiotic resistance marker genes. e) Protein characterisation was conducted in the single events and the GMO Panel was able to conclude on protein equivalence and food and feed safety. In the stack applications, bioinformatic analysis of proteins and open reading frames is conducted to predict similarity to toxins and allergens using updated databases, assessment of protein expression levels and proteins interactions. 5) The GMO Panel took note of the comment.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Hungary	Ministry of Agriculture	II.5 Environmental risk assessment	HU22	It is a misleading conclusion that MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize has no potential to cause adverse effects on human and animal health and the environment. The results presented in the Application do not support the safety of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize. 5.2.2.2: Statistically significant differences in forage composition were found between the MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize treated with herbicides and its comparator for calcium, moisture, phosphorus, ADF and NDF. Statistically significant differences in seed composition were found between the treated MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize and its comparator for alanine, aspartic acid, phenylalanine, serine, threonine, tyrosine, carbohydrates by calculation, glutamic acid, isoleucine, leucine, valine, protein, manganese, histidine and proline. Statistically significant differences in forage composition were found between the non-treated maize and its comparator for histidine, proline, histidine, proline, ADF and NDF. Statistically significant differences in seed composition were found between the non-treated maize and its comparator for alanine, aspartic acid, glutamic acid, isoleucine, leucine, linolenic acid, arachidic acid, eicosenoic acid, total fat, vitamin A, vitamin B6, vitamin E, carbohydrates by calculation, total dietary fiber, calcium, copper, iron, magnesium, manganese, ferulic acid, raffinose, proline and protein. 5.2.2.4: The results above clearly show that MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize treated or untreated with herbicides is clearly different from its comparator. 5.3.1-5.3.4: The presence of 6 cry toxins in the GM maize as well as that of an untested mixture of herbicide residues and metabolites was not considered. 5.3.5: This section does not mention the combined use of an	The GMO Panel assessed all significant differences between the six-event stack maize and its non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM maize reference varieties. None of the differences identified in forage and grain composition between the six event stack maize and the non-GM comparator needed further food/feed safety assessment except for the changes in levels of ADF in forage and protein, arginine, glycine, leucine, lysine and manganese in grain. The relevance of these changes were further discussed in Section 3.4.3. The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				untested cocktail of herbicides, while their residues and metabolites are present in food and feed. It does not take into account the possibility of herbicide drifts either. 5.3.7 : Human and animal health is clearly affected by the residues and metabolites of an untested mixture of herbicides. Therefore a careful monitoring of herbicide residue- and metabolite levels of every shipment of GM maize arriving to the EU is advised to be carried out. The effects of the untested herbicide residue- and metabolite level on soil microflora were not considered.	The risk assessment of herbicide residues in GM plants is not in the remit of the GMO Panel, but is performed by EFSA's Pesticides Unit. The GMO Panel considers that taken together, all the data in the dossier are sufficient to conclude on the absence of interactions between the events (including the newly expressed proteins) that would raise safety concerns in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.
Hungary	Ministry of Agriculture	II.6 Post-Market Environmental Monitoring Plan (PMEM)	HU23	The environmental monitoring plan stops monitoring at the point of food/feed or ingredients are produced. No monitoring was carried out by independent observers and although the questionnaires are filled by operators, they are not available for inspection. The present methods used in post market monitoring are not suitable to identify any risks. Even if any effect was observed during monitoring, it would be impossible to tie that effect to any specific GM crop. Because of long-term and delayed effects, the time period for monitoring should be much longer than the period for authorisation. Routine monitoring is conducted as a precaution to detect unforeseen effects. It would be important to inspect if there ever been such effects detected by general monitoring before. Beside the existing networks (importers, traders, silo-managers) no operators further down the food and feed chain have been selected for the general surveillance such as medical doctors, e.t.c. To see long term unintended effects, monitoring should be carried on for a few decades after the authorisation period of a GMO had expired. Even if any effects were found, they would not be able to be tied to the use/production of any specific GMO [see also Amanor-Boadu, V., Amanor-Boadu, Y. (2002): A survey of post-marketing surveillance of potential human late health effects of genetically modified foods' initiatives: lessons for Canada's strategy. Centre for Surveillance Coordination, Health Canada, http://legacy.library.ucsf.edu/tid/pyf20i00]. Monitoring stops at the point of sale, so there is no way to follow the health and other effects of GM food/feed on the	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				consumers.	
Hungary	Ministry of Agriculture	II.7 Additional information related to the safety of the genetically modified food or feed	HU24	References to Monsanto Technical Reports are not advantageous, since not everyone have access to those publications to judge the risks posed by a GMO. Also these reports are not considered as scientific publications.	The GMO Panel took note of the comment.
Hungary	Ministry of Agriculture	Part II – Scientific information	HU1	Hungary has objected to the authorisation of the maize events MON 87427, MON87460, MON89034, 1507, MON 87411 and 59122 and the stacks formed from them. Since all scientific objections still stand, Hungary very strongly objects to the authorisation of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize, even more so, since this stack expresses the proteins CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1, Cry35Ab1 proteins and the DvSnf7 dsRNA, and being sprayed with both glyphosate which has been shown to be genotoxic [Woźniak, E., Sicińska, P., Michałowicz, J., Woźniak, K., Reszka, E., Huras, B., Zakrzewski, J. & Bukowska, B. (2018): The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessment. Food and Chemical Toxicology 120. https://www.sciencedirect.com/science/article/pii/S0278691518304800] and glufosinate. It also contains an untested cocktail of their residues and metabolites, effects of which cannot be predicted. Evidence exists that chemicals, such as pesticides have a different effect from that of the individual chemicals if they are used in combination as mixtures or as individual chemicals over time, using different sequences of pesticides applications [Ashauer, R., O'Connor, I. & Escher, B. I. (2017): Toxic Mixtures in Time - The Sequence Makes the Poison. Environmental Science & Technology 51. https://pubs.acs.org/doi/10.1021/acs.est.6b06163]. In addition, this stack contains an ARM gene, the NptII, and the use of ARMs is discouraged in the EU. Herbicide resistant crops are less healthy for the consumers since they contain residues and metabolites of all the herbicide(s) applied to them. In addition, they contaminate the environment. It is possible to study the combined effect of glyphosate and glufosinate, their residues and metabolites experimentally, but those studies have not been done, and the	The GMO Panel assessed all six single maize events, the two-event stacks 1507 × 59122, 1507 × MON 89034, 1507 × MON 87427, 59122 × MON 89034, 59122 × MON 87427, MON 89034 × MON 87460, MON 89034 × MON 87427, MON 89034 × MON 87411, MON 87460 × MON 87427 and MON 87427 × MON 87411, the three-event stack 1507 × 59122 × MON 89034, 1507 × 59122 × MON 87427, 1507 × MON 89034 × MON 87427, 59122 × MON 89034 × MON 87427, MON 89034 × MON 87460 × MON 87427, MON 89034 × MON 87427 × MON 87411 and the four event stack 1507 x 59122 x MON 89034 x MON 87427 have been previously assessed by the GMO Panel (see Table 2 in the Scientific Opinion) and no safety concerns were identified. The risk assessment of herbicide residues in GM plants is not in the remit of the GMO Panel, but is performed by EFSA's Pesticides Unit. The GMO Panel considers that taken together, all the data in the dossier are sufficient to conclude on the absence of interactions between the events (including the newly expressed proteins) that would raise safety concerns in maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122. The EFSA GMO Panel assessed in previous opinions the probability and potential adverse effects of HGT of the recombinant DNA for the single events (see Table 1 in the Scientific Opinion) including the case of MON 87460 (EFSA GMO Panel, 2012; 2019b. No concern as a result of an unlikely, but theoretically possible, HGT of the recombinant genes to bacteria in the gut of domesticated animals and humans fed GM material or other receiving environments was identified.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
				outcomes cannot be predicted. The PAT and EPSPS enzymes are active in the microbes living in the human and animal gastrointestinal tract. Glyphosate is a selective antimicrobial agent (see United States Patent 7,771,736 (2010) Abraham, Assignee Monsanto: http://1.usa.gov/1IEMmWz https://www.google.com/patents/US7771736), therefore any residue will also have an effect on the gut microbiome of the consumers. These facts have not been taken into consideration by the applicant. The interaction between the different cry- and cry-type proteins in combinations on the hosts has never been studied. Therefore, the safety of MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize cannot be guaranteed and should not be allowed to reach the EU market.	
Italy	Ministry of Environment	II.5 Environmental risk assessment	Italy - 1 Environmental Risk Assessment (ERA)	The application of the approach for risk assessment is not carried out correctly, on the basis of the provisions of the legislation and the EFSA guidances. Indeed, the notifier conclude that the risk is negligible already in the first step "problem formulation", and consequently affirm that it is not necessary to further deepen the other steps. We suggested to review the environmental risk assessment, for this and for the other areas of risk, relevant to this notification	The GMO Panel took note of the comment. The GMO Panel concluded that it is unlikely that maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 would differ from conventional maize varieties in its ability to persist under EU environmental conditions. Considering the scope of application EFSA-GMO-NL-2017-139, interactions of occasional feral six-event stack maize plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of horizontal gene transfer from the six-event stack maize to bacteria does not indicate a safety concern. Therefore, considering the combined traits, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that the six-event stack maize would not raise safety concerns in the event of accidental release of viable GM maize grains into the environment.
Italy	Ministry of Environment	II.6 Post-Market Environmental Monitoring Plan (PMEM)	Italy - 2 Post-Market Environmental monitoring plan (PMEM) - Approach	Approach: In the paragraph it is stated that "The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 maize". In order to better evaluate the proposed general surveillance plan it is required to provide the content of the above mentioned guidance because it is right during the handling of goods that unintended release into the environment can occur.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Italy	Ministry of Environment	II.6 Post-Market Environmental Monitoring Plan (PMEM)	Italy - 3 Post-Market Environmental monitoring plan (PMEM) - Existing systems	The authorization holder is working together with other members of the plant biotechnology industry within the European Association of Bioindustries (EuropaBio) and trade associations representing the relevant operators in order to implement an harmonised monitoring methodology. Not all Member States are represented within these associations: therefore, it would be appropriate to provide explanations on the monitoring methodology adopted in the MS not represented.	Monitoring is related to risk management, and thus a final adoption of the post-market environmental monitoring (PMEM) plan falls outside the mandate of EFSA. The GMO Panel considered that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122.
Italy	Ministry of Environment	II.6 Post-Market Environmental Monitoring Plan (PMEM)	Italy - 4 Post-Market Environmental monitoring plan (PMEM) - Monitoring Methodology	In the EFSA guidance on PMEM (EFSA Panel on Genetically Modified Organisms, 2011) is established that applicants should provide raw data: then, it would be appropriate to have these data available	The GMO Panel took note of the comment.
Netherlands	Dutch GMO Office	Part II – Scientific information	Dutch comment on EFSA/GMO/NL /2017/139 2	The Dutch CA has assessed the dossier with respect to the food and feed safety of MON87427 x MON87460 x MON89034 x 1507 x MON87411 x 59122 maize and has no comments or requests for additional information in relation to the safety of this GM event.	The GMO Panel thanks the Dutch Competent Authority for this comment.
Netherlands	Dutch GMO Office	Part II – Scientific information	Dutch comment on EFSA/GMO/NL /2017/139	The applicant claims that all the information in the application is confidential. Information which is crucial to assess potential risks of a GM crop should not be declared confidential, because a lack of transparency undermines public trust in the risk assessment. This is in conflict with the Aarhus Convention, which regularises the right of the public to access environmental information and has been implemented in the European legislation. According to Article 30 of Regulation (EC) No 1829/2003 information on amongst others the composition of a GMO, physico-chemical and biological characteristics, and effects on human and animal health and the environment cannot be declared confidential. The Dutch CA on Regulation (EC) No 1829/2003 will send an email on this matter to the European Commission.	EFSA and its GMO Panel based the scientific risk assessment of maize maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 on a comprehensive information package that was made of the valid application EFSA-GMO-NL-2017-139 (including confidential and nonconfidential information and data), additional information provided by the applicant during the risk assessment, relevant scientific comments submitted by the Member States and relevant peer-reviewed scientific publications. In parallel, applicants submit their claims to treat element(s) of their applications as confidential business information to the European Commission and this is up to the European Commission to assess the validity of these claims.
Norway	VKM	II.1 Hazard identification and characterisation	Norwegian Scientific Committee for Food and Environment (VKM)_1	VKM questions whether there is sufficient knowledge on the safe use of RNAi in GM-plants.	EFSA is aware of the particularities that the risk assessment of RNAi-based GMPs can pose. EFSA has taken several actions to determine whether the existing risk assessment approaches for GMPs are appropriate for the risk assessment of RNAi based GMPs or require complementary or alternative approaches. An overview of EFSA's activities on the risk assessment of RNAi-based GMPs is given in Papadopoulou et al. (2020)

Country	Organization	Reference	Topic	Comment	EFSA GMO Panel responses
Norway	VKM	II.1.3.3 Selection of material and compounds for analysis	Norwegian Scientific Committee for Food and Environment (VKM)_4	VKM welcomes information on herbicide residue levels and their relevant metabolites in applications for herbicide tolerant GM-plants. Data on glyphosate and glufosinate-ammonium residue levels, including their relevant metabolites, in plant material from field studies would support the assessment of food and feed safety.	The GMO Panel thanks the Norvegian Competent Authority. The risk assessment of herbicide residues in GM plants is not in the remit of the GMO Panel, but is performed by EFSA's Pesticides Unit.
Norway	VKM	II.1.4.1 Testing of newly expressed proteins	Norwegian Scientific Committee for Food and Environment_ 2	The applicant states: "The CP4 EPSPS, CspB, NptII, Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins and the DvSnf7 dsRNA have no synergistic or antagonistic effects to each other. The modes of biological activity are different for these proteins and there is no known or conceivable mechanism of interaction between all proteins which could lead to adverse health effects in animals or humans." VKM opposes since this statement is not supported by any experimental data. Different modes of action do not necessarily exclude interaction.	The GMO Panel took the comment into account. See Section 3.4.3.3 for details on the assessment of possible interactions of relevance for food and feed safety.
Norway	VKM	II.1.4.4 Testing of the whole genetically modified food or feed	Norwegian Scientific Committee for Food and Environment_ 3	VKM would have preferred that the applicant performed a nutritional feeding study on the stacked event with animals fed a high inclusion of maize gluten in their diets.	The nutritional assessment of maize MON 87427 × MON 87460 × MON 89034 × 1507 × MON 87411 × 59122 was conducted on the basis of the comparative analysis. The biological relevance of compounds showing differences, the role of maize as contributor to their total intake and the magnitude and direction of the observed changes does not represent concern which would require nutritional feeding studies in animals.
Spain	Comisión Nacional Bioseguridad	II.1.4 Toxicology	CNB comment on Toxicology and Exposure assessment	Regarding studies carried out on "Toxicology and Exposure assessment", the applicant reports on the high exposure margin (MOE) obtained from acute toxicity studies, as a safety justification for these GMOs. From our point of view, we consider this approach might be wrong, first of all because acute toxicity studies add little value to the risk assessment for the repeated consumption of food or feed derived from genetically modified plants, but also because the NOAEL that must be used to make the risk characterization and establishing a MOE, is a NOAEL derived from a repeated dose toxicity study, not from an acute toxicity study in which the administration of the product is done only once. From our point of view, and in order to have a complete safety assessment of these GMOs, it would be desirable to have a 90-day study with the complete food/feed.	The GMO Panel took note of the comment. Animal studies on food/feed derived from the six event stack maize are not necessary, based on the outcome of the molecular characterisation, comparative analysis and toxicological assessment (see Section 3.4.3.3 of the Scientific opinion for further details).

Note: For the full reference of the publications cited in the GMO Panel responses, please see the reference list of the Scientific Opinion. For the publications cited only in this document, a full reference is provided as a link in the responses or below.

EFSA (European Food Safety Authority), 2017. Outcome of the public consultation on the draft guidance on allergenicity assessment of genetically modified plants. EFSA supporting publication 2017: 14(6):EN-1259. 98 pp. doi: 10.2903/sp.efsa.2017.EN-1259

EFSA (European Food Safety Authority), Álvarez F, Devos Y, Georgiadis M, Messéan A, Waigmann E. Annual post-market environmental monitoring report on the cultivation of genetically modified maize MON 810 in 2016. EFSA J. 2018;16(5):e05287. Published 2018 May 25. doi:10.2903/j.efsa.2018.5287

EFSA (European Food Safety Authority), Paraskevopoulos K, Ramon M, Dalmay T, du Jardin P, Casacuberta J, Guerche P, Jones H, Nogué F, Robaglia C, Rostoks N 2018. Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation. EFSA supporting publication 2018: 15(8):EN-1466. 13 pp. doi: 10.2903/sp.efsa.2018.EN-1466

Papadopoulou N, Devos Y, Álvarez-Alfageme F, Lanzoni A, Waigmann E, 2020. Risk Assessment Considerations for Genetically Modified RNAi Plants: EFSA's Activities and Perspective. Front Plant Sci. 2020 Apr 21;11:445. doi: 10.3389/fpls.2020.00445.