Application EFSA-GMO-NL-2016-131 (maize MON 87427 x MON 89034 x MIR162 x NK603) **Comments and opinions submitted by Member States during the three-months consultation period**				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Organization**	**Reference**	**Comment**	**GMO Panel response**
Austria	Fed.Ministry_Health/Women's Aff.	II.1 Hazard identification and characterisation	Detection method: Providing an event specific detection method for each parental line and a maize specific reference PCR system is not satisfactory. Generally, a validated event specific detection method for this stacked event MON87427xMON89034xMIR162xNK603 should be presented before deciding about the placing on the market of this product. The detection method for GM maize MON87427xMON89034xMIR162xNK603 was sent for validation to CRL. The current evaluation status of the method is "Step 2 (scientific assessment) ongoing" (http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx).	This issue is not in the remit of the GMO Panel.
Austria	Fed.Ministry_Health/Women's Aff.	II.1 Hazard identification and characterisation	General comment: In his assessment of GM maize MON87427xMON89034xMIR162xNK603 the notifier refers to the previous assessments conducted for the individual events combined into GM maize MON87427xMON89034xMIR162xNK603 instead of presenting specific data for the stacked event in question. The assessment also focuses on the direct effects of the transgenic proteins expressed by GM maize MON87427xMON89034xMIR162xNK603 and does not sufficiently address unintended effects associated with GM maize MON87427xMON89034xMIR162xNK603. As a justification the notifier claims that unintended effects of the modifications contained in GM maize MON87427xMON89034xMIR162xNK603 have already been addressed in the frame of the previous assessments conducted for the events used to generate GM maize MON87427xMON89034xMIR162xNK603. Based on our comments submitted to EFSA concerning these assessments we cannot fully agree with this approach. We request that the notifier is asked to provide appropriate information to address any open questions as regards	The GMO Panel took note of the general comment. The specific comments are addressed below.

			unintended effects associated with events combined into GM maize MON87427xMON89034xMIR162xNK603 or GM maize MON87427xMON89034xMIR162xNK603 itself (see our comments below).	
Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.2 Information relating to the genetically modified plant	1.2.2.3 Information on the expression of the inserted/modified sequence: Scientific Information, p. 26: The applicant maintains that "there is no known mechanism by which glyphosate application to GM maize MON87427xMON89034xMIR162xNK603 could affect protein expression levels in this product." This is not quite correct considering the fact that glyphosate is abolishing protein synthesis in sensitive organisms (Chekan et al. 2016). Glyphosate is modulating the phosphoenolpyruvate conversion rate of the 5-enolpyruvylshikimate-3-phosphate synthase (compare Part II/Scientific Information - EFSA-GMO-DE-2016-130). By reducing the pool of aromatic amino acids due to a reduction of precursor molecules necessary for the synthesis of phenylalanine, tryptophan and tyrosine the overall protein synthesis rate of a cell is affected (Chekan et al. 2016). This is obvious for the plant-specific EPSPS protein under glyphosate exposure which is inhibited by glyphosate and terminates protein synthesis (Schönbrunn et al. 2001). But also - albeit to a lesser extent - the phosphoenolpyruvate turnover rates for genetically modified bacterial versions of EPSPS are affected by glyphosate interfering with overall protein synthesis (compare Part II/Scientific Information - EFSA-GMO-DE-2016-130). Therefore, glyphosate is indeed affecting "protein expression levels" although - probably - not directly via interaction with genetic regulatory elements (i.e. by influencing the "gene" expression) but indirectly via interference with the pool of available aromatic amino acids. [Chekan JR, Cogan DP, Nair SK, 2016. Molecular basis for resistance against phosphonate antibiotics and herbicides. MedChemComm 7(1): 28-36. Schönbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrhein N, Evans JNS, Kabsch W, 2001. Interaction of the	The GMO Panel took note of the comment.

			herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. Proc Natl Acad Sci U S A 98(4): 1376-1380.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.2 Information relating to the genetically modified plant	1.2.2.4 Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant: The notifier bases his assessment of genetic stability on conclusions from the assessment of the parental GM events combined in GM maize MON87427xMON89034xMIR162xNK603 and on deliberations concerning the mechanism of homologous recombination in plants. We take note that the assessment is not based on data established for GM maize MON87427xMON89034xMIR162xNK603 and does not further address our concerns as regards the previous assessment of genetic stability of the parental GM events. Therefore, we do not consider that the conclusions taken by the notifier are sufficiently robust.	Data on the genetic stability over several generations have been provided in the single applications and has been assessed by the GMO Panel. Furthermore, sequence analysis of the MON 87427 x MON 89034 x MIR162 x NK603 showed that the inserts have retained their integrity. In addition, the GMO Panel considers that there is very low likelihood for the insert sequences becoming more unstable when combined together by traditional breeding.
Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.2 Information relating to the genetically modified plant	1.2.2.3 Information on the expression of the inserted/modified sequence: For the assessment of the expression of the transgenic inserts in GM maize MON87427xMON89034xMIR162xNK603, levels of transgenic proteins in GM maize MON87427xMON89034xMIR162xNK603 were presented for grain (R6) and forage (R5) (Mozaffar et al. 2013a). Test material was produced in a field trial at 5 sites in the USA in 2013, using a randomised, complete block design with 4 replicates per site. Protein levels were analysed across sites and means, standard deviations and data ranges are presented in the dossier (c.f. Scientific Information, Tab. 2-6, p. 27-31). However, further information is required to sufficiently assess transgene expression in a range of conditions: • The notifier should provide a rationale for the selection of the different test sites as well as further evidence for the representativeness of test sites for geographic regions used for commercial production of GM maize	Based on the USDA map of corn acreage (http://www.nass.usda.gov/Charts_and_Maps/Crops_County/cr-pl.php), it can be concluded that field trials were carried out in representative maize-growing areas. In addition, information on

			MON87427xMON89034xMIR162xNK603. A sound justification according to existing guidance (EFSA 2010) should be provided to indicate that the field trial sites cover a range of environmental and agronomic conditions as experienced during commercial production of maize taking into account differences between cultivation years. The criteria used by the notifier for choice of locations should be adequately discussed in the technical dossier. • The notifier should also indicate whether the agricultural management of the field sites corresponds to agricultural procedures commonly used for commercial production of maize. This consideration should take into account changes in agricultural management which can be expected for GM maize MON87427xMON89034xMIR162xNK603 in the next 10 years, such as e.g. increased amounts of Glyphosate-herbicides used for crop treatments and increased frequency of individual treatments which are usually the first measures to counter weed resistance to the standard application regime for Glyphosate-based herbicides. The notifier also did not submit transgene expression data for GM maize MON87427xMON89034xMIR162xNK603 not treated with Glyphosate-based herbicides. Thus CP4 EPSPS expression levels are only presented for Glyphosate-treated GM maize MON87427xMON89034xMIR162xNK603 and the Glyphosate tolerant parental events and data for CRY proteins expressed in Glyphosate-treated GM maize MON87427xMON89034xMIR162xNK603 is compared with data for untreated GM maize MON89034. We request that the notifier provides further information on the above mentioned aspects and a more robust data basis that allows an appropriate assessment of expression of the transgenes under various environmental conditions. If no justification can be provided to establish the representativeness of the field trial as regards the geographic regions and the range of receiving	the five field sites used for the protein expression analysis is included in the report of the phenotypic evaluation and environmental interactions of maize MON 87427 × MON 89034 × MIR162 × NK603 (Report no: MSL0027656). Overall, the GMO Panel considers this information sufficient. The GMO Panel acknowledges that plants of some of the single events were treated differently compared to the four-event stack maize MON 87427 × MON 89034 × MIR162 × NK603. However, protein levels from plant material mainly used for food/feed purposes such as grain and forage are determined in sprayed plants, which are representative of the commercially growing conditions for the four-event stack maize. In addition, the GMO Panel considered that: i) expression levels in both treated and untreated plants of either the four-event stack maize or the single events are similar except for expected changes in the levels of CP4 EPSPS resulting from the combination of MON 87427 and NK603 single events, both producing CP4 EPSPS protein in the four-event stack maize and ii) there is no hypothesis of a mechanism of interaction (also considering the possible effects of the herbicide) actually occurring that could significantly change the levels of the newly expressed proteins (NEPs). Based on the above, the GMO Panel is of the opinion that the data on the expression levels of the NEPs is appropriate to conclude that there are no interactions between the events that would affect protein expression levels in maize MON 87427 × MON 89034 × MIR162 × NK603.

			environments for commercial production of maize, additional data for more than one growing season at the same locations should be provided. Furthermore the between-site variation should be analysed to account for interactions with the respective environment (gene x environment interactions).	The analysis of protein expression data was done in line with EFSA GMO Panel (2011a).
Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.2 Information relating to the genetically modified plant	1.2.2.5 Potential risk associated with horizontal gene transfer: Scientific Information, p. 35: The applicant refers to homology requirements necessary for recombination between plant and bacterial DNA and maintains that "the donating plant and recipient bacteria must share at least two 70 bp of DNA sequences having at least 67 identical nucleotides" and that "these two homologous regions must flank the ends of a "gene" in the transgene of the plant genome." We appreciate that the applicant has tried to evaluate the possibility of gene transfer from the transgenic insert in the plant genome to bacterial recipients by performing in silico analyses (i.e. homology searches) for potential recombination sites in microbial genomes. The approach as presented by the applicant may provide some broad overview about the general recombination potential of the involved sequences. However, this methodology is too insensitive to provide relevant results concerning the potential for homologous recombination for the following reasons: 1) We would like to remind the applicant that the minimum efficiently processed segment (MEPS) lengths (i.e. mismatch-free, 100% sequence identity between donor and recipient strand) for homologous recombination are between 23 and 27 bp (Shen and Huang 1986) and usually approx. 26 bp (Majewski and Cohan 1999). In Bacillus short regions of complete identity on both ends of the intruding stands are necessary but elements as short as 20 bp with complete homology are sufficient to initiate ssDNA invasion, strand displacement and homologous recombination (Majewski and Cohan 1999). 2) A sequence identity between transgenic and wild type	The potentials and limitations of short sequences for facilitating homologous recombination have been considered by EFSA when setting up the criterion of 200 bp length (see EFSA, 2017b). The applicant has followed these recommendations for conducting their HGT risk assessment. It is true that some uncertainty in the HGT risk assessment remains, because of the theoretically possible involvement of shorter sequences for facilitating homologous recombination and also because the databases do not harbour all DNA sequences of all existing microorganisms. However, in addition to the consideration of the likelihood of recombination, the risk assessment also includes the identification of potential hazards caused by the transfer of the genetic elements of bacterial origin from the GM plants to environmental bacteria. In case of the genes used for this GM maize, no selective advantage would be conferred to recipients. It is unlikely that the plant codon optimised genes would perform better in bacteria than the

			bacterial sequences below 100% only reduces the rate of recombination but does not exclude homologous recombination: The rate of homologous recombination is decreasing in a log-linear relationship (Zawadzki et al. 1995) with increasing sequence divergence among the involved DNA molecules and falls below the level of detection at a sequence divergence higher than 25-30% (or a sequence identity below 70-75%) (Rao et al. 1995; Fraser et al. 2007). 3) The applicant is dismissing the possibility for homologous recombination mediated by homology-directed illegitimate recombination relying only on an anchor sequence (with a high grade of homology to the target sequence) and a very short region of micro homology (approx. 3-10 bp) at the opposite end of the intruding strand (de Vries and Wackernagel 2002; Prudhomme et al. 2002). In combination with the information described under point 1 the threshold of 70 bp of homology (i.e. sequence identity) is, thus, irrelevant for a large number of occasions for recombination occurring under real life conditions in natural environments (soil, gastrointestinal tract, etc.). The frequency of homology-facilitated illegitimate recombination is several orders of magnitude lower compared to the rate mediated by homologous recombination. However, for the soil bacterium Acinetobacter BD413 the minimum length of the required anchor sequence was determined to be >183 bp, the region of micro homology was 3-8 bp and frequency of strand exchange was still 0.01% of the rate for homologous recombination (de Vries and Wackernagel 2002). The applicant is also requested to provide a scientific rationale for selecting 70 bp as a threshold for the minimal length of alignment. The applicant states that "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." It should be noted that recombination does not necessarily result in all cases in the deletion of host DNA and that fitness costs imposed by HGT events on the receptor bacterial cells may be easily compensated (Michod et al. 2008; Didelot and Maiden 2010; Moradigaravand and Engelstadter 2012; Paul et al. 2013).	respective bacterial genes which already occur in the environment. 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. In addition, because of plant codon optimisation, the recombinant gene products are probably less functional than their natural variants in bacterial cells. The GMO Panel took note of the comments raised by Austria

			The applicant states that "the gene transferred from the plant genome must provide an advantage to the recipient bacteria in the environment over its untransformed neighbors." This is the case for soil or plant-associated bacteria sensitive to glyphosate. By acquiring an epsps gene mediating tolerance to glyphosate the transformed bacteria have a significant growth advantage over their untransformed neighbors in environments under glyphosate exposure (i.e. fields with MON87427, MON89034, MIR162 and NK603 cultivation). Maize fields under glyphosate treatment mediate a significant selection pressure on exposed soil and plant-associated bacteria.	As reported above, natural variants of the bacterial genes, including *epsps*, exist in the environment and the likelihood of their HGT is much higher than for the transfer from GM plants to bacteria. In addition, because of plant codon optimisation, the recombinant gene products are probably less functional than their natural variants in bacterial cells
			Some of the most pronounced effects of glyphosate on bacterial communities are documented below: The application of glyphosate is an integral part during the life cycle of MON87427, MON89034, MIR162 and NK603. Therefore, soil and plant-associated bacteria are expected to be exposed repeatedly to this herbicide. Mammalian gut bacteria may be exposed to this herbicide by feed containing transgenic plants with residues of glyphosate. Glyphosate application mediates substantial selection pressure and perturbations on exposed bacterial populations (Araujo et al. 2003; Kremer and Means 2009). Glyphosate was shown to be toxic to bacteria grown in vitro in liquid media (Busse et al. 2001). The microbial activity changed/increased in soil bacterial populations exposed to glyphosate (Busse et al. 2001; Araujo et al. 2003). Glyphosate exerted "selection pressure" on Bacillus japonicum (Arango et al. 2014). Considerable effects of glyphosate on oxygen uptake and respiration of soil bacteria were reported (Roslycky 1982; Lane et al. 2012). A substantial change in the bacterial community was observed after the application of high doses of glyphosate to clay loam and sandy forest soils (Ratcliff et al. 2006). Glyphosate induced shifts in the rhizosphere community of Roundup Ready soybeans (Arango et al. 2014). Glyphosate induced shifts in the endophytic bacterial communities of RR soybeans (Arango et al. 2014). Specific bacterial species (Burkholderia sp.) were inhibited by glyphosate whereas other species (Gemmatimonas sp.) were promoted (Arango et al. 2014). Glyphosate is a substrate for some micro-organisms and, thus, promotes their growth (= positive	This issue is not in the remit of the GMO Panel.

			selection) (Kuklinsky-Sobral et al. 2005): "The resilience of the soil after glyphosate selection pressure could include a succession of glyphosate metabolizing bacteria, accomplished by rapid bacterial adaptation by mutations and bacterial growth changes " (Arango et al. 2014). Glyphosate is suspected to have been the causative agent for the increased risk of Clostridium botulinum infections in cattle in Germany over the past decade (Krüger et al. 2013). Glyphosate may alter the community structure of gut bacteria in poultry resulting in a reduction of the beneficial and a promotion of pathogenic bacteria (Shehata et al. 2013). "The repeated use of glyphosate may create a selection pressure in soil microbial communities " (Lane et al. 2012). Negative impacts of glyphosate have been observed in other studies on specific microbial groups inhabiting glyphosate-resistant plant rhizospheres (Kremer and Means 2009; Barriuso et al. 2010; Zobiole et al. 2011) and on gram negative bacteria after repeated applications of the herbicide in microcosms (Lancaster et al. 2010). Complete growth inhibition was reported for Bacillus mycoides and an unidentified strain isolated from soil at the lowest applied glyphosate concentration (commercially available products: Roundup Quick, Roundup Max, and isopropyl amine salt of glyphosate) (Sihtmäe et al. 2013). In summary, these observations are indicative for a certain biologically relevant effect on the viability and the community structure of exposed soil, gut, and plant-associated bacteria after exposure to glyphosate containing herbicides. The lateral transfer of epsps/aroA or pat genes or fragments thereof is therefore of significance taking into account the long-term and large-scale application of the respective herbicide. This situation exerts variable levels of selection pressure in favor of an acquisition of such genetic elements for many constituents of an exposed soil bacterial community. We would like to ask the applicant to at least consider and discuss these observations for an appropriate risk assessment of lateral gene transfers from transgenic plants to bacteria. [Arango L, Buddrus-Schiemann K, Opelt K, Lueders T, Haesler F, Schmid M, Ernst D, Hartmann A, 2014. Effects of	HGT considers the transfer of genetic elements which would be functional in recipients and provide a selective advantage. The transfer of DNA fragments could become relevant if connected to a selective advantage; however, in the assessment of the four-event stack maize, no hypothesis was found that this could be the case. The natural variants of the mentioned bacterial genes exist in the environment and the likelihood of their HGT is much higher than for the transfer from GM plants to bacteria. In addition, because of plant codon optimisation, the recombinant gene products are probably less functional than their natural variants in bacterial cells (see Scientific Opinion, Section 3.3.4.2).

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Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.2 Information relating to the genetically modified plant	1.2.2.5 Potential risk associated with horizontal gene transfer: Scientific Information, p. 34: The applicant maintains that it is highly unlikely that plant-derived DNA recombines with genomic DNA in human and animal cells and refers to ”the existence of natural barriers that inhibit the cellular uptake of exogenous DNA” and states that ”each of these barriers would serve to limit and/or eliminate the availability of exogenous DNA that might be capable of recombination with human or animal cells.” We would like to indicate that orally ingested DNA is not completely degraded during its passage through the gastrointestinal tract as the applicant conveys the impression. DNA from dietary sources is present throughout the animal gastrointestinal tract and according to their	The GMO Panel took note of the comments raised by Austria and wishes to clarify that besides exposure it also considered the consequences of an unlikely but theoretically possible HGT. The updated bioinformatics analyses of events MON 87427, MON 89034, MIR162 and NK603 do not reveal any new DNA

			lengths and initial copy number a small amount of DNA fragments survive the passage and is detectable in excreted faeces (Schubbert et al. 1994; Schubbert et al. 1997; Schubbert et al. 1998; Einspanier et al. 2001; Hohlweg and Doerfler 2001; Chowdhury et al. 2004). DNA from the gut lumen is transferred to the nuclei of cecal epithelial cells to some extent (Palka-Santini et al. 2003). Orally administered test DNA is detectable in blood, spleen, liver and kidney of animals (Schubbert et al. 1994; Schubbert et al. 1997; Schubbert et al. 1998; Hohlweg and Doerfler 2001). Recombinant DNA was detected in the blood of the test animals fed with transgenic plants (Tudisco et al. 2010). In a natural scenario (i.e. food/feed application) plant-derived DNA was found in liver and spleen samples of the fed animals (Hohlweg and Doerfler 2001) or in their blood (Bertheau et al. 2009). After the consumption of rabbit meat rabbit-specific DNA was found in the cellular and the plasma compartment of blood from human consumers (Forsman et al. 2003). Chromosomal linkage of orally administered test DNA was observed in a mouse model (Schubbert et al. 1998). Recombinant DNA was detected in milk after feeding transgenic plant material (Agodi et al. 2006; Tudisco et al. 2010) and in organ/tissue samples of the tested animals (Alexander et al. 2007; Sissener et al. 2010). By referring to the literature data presented above we would like to point to the fact that there is no absolute barrier against the uptake of foreign food/feed-borne DNA (including recombinant DNA) into peripheral blood, cells or tissues of animals. Transfer of plant-derived DNA (including recombinant DNA) from the alimentary tract to eukaryotic organs is possible but strictly dependent on fragment lengths, copy number and the sensitivity of the applied detection systems. We would like to ask the EFSA GMO Panel to take these observations into consideration. The applicant refers to the publication of Netherwood et al. and maintains that "no evidence was found to suggest gene transfer between GM maize and intestinal micro-flora occurred during the feeding experiments." Netherwood et al. have used GM soya in their experiments and, thus, were incapable to produce scientific data on GM maize. Moreover, the applicant does not mention that Netherwood et al. explicitly state that "three of seven ileostomists	sequence that could provide sufficient length and identity which could facilitate HGT by double homologous recombination, confirming the previous conclusions (EFSA GMO Panel, 2017a,b, 2019a,b,c). In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from event MON 87427, MON 89034, MIR162 and NK603 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT. As mentioned above, an updated bioinformatic analysis has been conducted by the applicant in line with the latest EFSA requirements. Some uncertainty in the HGT risk assessment remains, because of the theoretically possible involvement of shorter sequences for facilitating homologous recombination and also because the databases do not harbour all DNA sequences of all existing microorganisms. However, in addition to the consideration for the likelihood of recombination, the risk assessment also includes the identification of potential hazards caused by the transfer of the genetic elements of bacterial origin from the GM plants to environmental bacteria. In the case of maize MON 87427 × MON 89034 × MIR162 × NK603 it is unlikely that an unlikely but theoretically possible HGT will confer a selective advantage to recipients.

			showed evidence of low-frequency gene transfer from GM soya to the microflora of the small bowel before their involvement in these experiments " (Netherwood et al. 2004). This is indeed an indication for the possibility of epsps gene transfer from plant to bacteria in natural habitats of exposed bacteria. The applicant is referring to an overall complexity and low probability which would make horizontal gene transfer between plant and bacteria "unlikely " and states that "the recipient bacteria must be competent and able to accept exogenous DNA " implicating their absence in the environments under consideration. We would like to indicate that a huge number of bacterial species harbor competence genes (e.g. gamma-proteobacteria (Cameron and Redfield 2006). However, competence induction is a fine tuned process relying usually on environmental stimuli (including intercellular signaling, stress response, and nutrient starvation among others) (Seitz and Blokesch 2013). The exact environmental cues inducing competence in a given species remain to be determined, yet, for the majority of bacterial species. But that does not mean that most bacteria are not capable to enter a competent state. Absence of evidence is not evidence for absence (of competent bacteria). [Agodi A, Barchitta M, Grillo A, Sciacca S, 2006. Detection of genetically modified DNA sequences in milk from the Italian market. Int J Hyg Environ Health 209(1): 81-88. 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. 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. Cameron AD, Redfield RJ, 2006. Non-canonical CRP sites control competence regulons in Escherichia coli and many	

			other gamma-proteobacteria. Nucleic Acids Res 34(20): 6001-6014. Chowdhury EH, Mikami O, Murata H, Sultana P, Shimada N, Yoshioka M, Guruge KS, Yamamoto S, Miyazaki S, Yamanaka N, Nakajima Y, 2004. Fate of maize intrinsic and recombinant genes in calves fed genetically modified maize Bt11. J Food Prot 67(2): 365-370. Einspanier R, Klotz A, Kraft J, Aulrich K, Poser R, Schwagele F, Jahreis G, Flachowsky G, 2001. The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. Eur Food Res Technol 212(2): 129-134. Forsman A, Ushameckis D, Bindra A, Yun Z, Blomberg J, 2003. Uptake of amplifiable fragments of retrotransposon DNA from the human alimentary tract. Mol Genet Genomics 270(4): 362-368. Hohlweg U, Doerfler W, 2001. On the fate of plant or other foreign genes upon the uptake in food or after intramuscular injection in mice. Mol Genet Genomics 265(2): 225-233. 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. Palka-Santini M, Schwarz-Herzke B, Hosel M, Renz D, Auerochs S, Brondke H, Doerfler W, 2003. The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol Genet Genomics 270(3): 201-215. 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. 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. Seitz P, Blokesch M, 2013. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. Sissener NH, Johannessen LE, Hevroy EM, Wiik-Nielsen CR, Berdal KG, Nordgreen A, Hemre GI, 2010. Zebrafish (Danio rerio) as a model for investigating the safety of GM feed ingredients (soya and maize); performance, stress response and uptake of dietary DNA sequences. Br J Nutr 103(1): 3-15. Tudisco R, Mastellone V, Cutrignelli MI, Lombardi P, Bovera F, Mirabella N, Piccolo G, Calabro 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(10): 1662-1671.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects	1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects 1.2.3.2 Any change to the ability of the genetically modified plant to transfer genetic material to other organisms: (b) Plant to plant gene transfer: The traits selected to observe "reproductive morphology" are not sufficiently accurate to conclude that the outcrossing potential is unchanged compared to conventional maize varieties. Whereas due to the greater exposure this would not be acceptable upon cultivation, it is tolerable given the exclusion of cultivation from the scope of the current application.	The GMO Panel took note of the comment.
Austria	Fed.Ministry_Health/Women's Aff.	II.1.3.2 Experimental design and statistical analysis of data from field trials	For the comparative assessment (compositional analysis as well as agronomic and phenotypic characteristics), GM maize MON87427xMON89034xMIR162xNK603 (treated as well as untreated with Glyphosate-based herbicides), a non-transgenic control and 17 conventional varieties were grown in a field trial conducted in the USA in 2013 at 8 sites, using a randomised, complete block design with 4	As discussed in Section 3.5.3. of the Scientific Opinion, the GMO Panel considers that the selected sites reflect commercial maize-growing regions in which the test materials are likely to be grown. It also considers that the meteorological dataset, including the extreme events, remains within the range of climatic conditions expected to occur at the selected sites.

		for comparative analysis	replicates per site (Scientific Information, p. 38ff.). However, some information important to assess the data from the comparative assessment in a range of conditions is not provided: • The information submitted as regards the cultivation conditions e.g. concerning data on climatic, ecological, soil and agronomic conditions is not sufficiently discussed, e.g. as regards the influence of crop treatments with pesticides on the outcomes of the performed analyses, specifically on the assessment of disease and pest damage. • The notifier did not assess whether the agricultural management of the field sites corresponds to usual agricultural procedures for commercial maize production. The notifier should provide all available data for analysis and submit further information on the abovementioned aspects.	Finally, the GMO Panel considers that the management practices of the field trial sites, including planting, harvesting and application of plant protection products are typical of receiving environments where the field trial sites were located.
Austria	Fed.Ministry_Health/Women's Aff.	II.1.3.4 Comparative analysis of composition	1.3.3 Compositional analysis: For the compositional analysis, material from the field trial discussed under Chapter 1.3.2 was used for analysis. However, some relevant information to assess the data provided by the notifier is missing (see comments on the experimental design of the field trials, 1.3.2) and no rationale is provided whether the sample size and the design of the field trial were sufficient to detect potential differences in composition for all parameters. The assessment falls short of demonstrating that the data basis is sufficient to assess the composition of GM maize MON87427xMON89034xMIR162xNK603 under a range of environmental conditions. We request that the notifier provides further information on the abovementioned aspects. Additionally, the amount of residues of the herbicide treatment should be assessed including amounts of herbicide metabolites present in the produced material. A recent review of compositional analyses and feeding studies conducted with herbicide tolerant crop material demonstrated the need to better take into account current production conditions for herbicide-tolerant crops in the design of field tests (Cuhra 2015). This is necessary to ensure that assessments are representative	As explained in detail in the responses above, the field trial design was in line with the applicable guidance documents (EFSA GMO Panel, 2011a, 2015a). The GMO Panel was able to conclude on the risk assessment based on the information provided by the applicant. The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel.

			of commercial cultivation conditions. Due to increasing weed resistance to glyphosate application rates and frequencies of application of glyphosate-herbicides are rising (Heap 2015; Benbrook 2016). The more frequent use and/or higher amounts of herbicides used in commercial cultures may lead to higher levels of herbicide residues and metabolites in harvested crop material (cf. Benbrook 2012; Benbrook 2016; Myers et al. 2016). We recommend that further analysis of this issue is requested. For this analysis the notifier should take into consideration that the CP4 EPSPS transgenes are expressed at a higher level in GM maize MON87427xMON89034xMIR162xNK603 which may affect the maximum level of Glyphosate herbicides that is used in the crop. [Benbrook C, 2012. Impacts of genetically engineered crops on pesticide use in the U.S. - the first sixteen years. Environmental Sciences Europe 24(1): 24. Benbrook CM, 2016. Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe 28(1): 1-15. Cuhra M, 2015. Review of GMO safety assessment studies: glyphosate residues in Roundup Ready crops is an ignored issue. Environmental Sciences Europe 27(1): 1-14. Heap I, 2015. The International Survey of Herbicide Resistant Weeds; www.weedscience.org; (last accessed: 04/11/2015). 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.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.1.3.4 Comparative analysis of composition	1.3.3 Compositional analysis: Statistically significances: The notifier included the GM maize MON87427xMON89034xMIR162xNK603, the conventional counterpart (LH244xLH287), and 17 references maize	The GMO Panel thanks Austria for this summary.

			varieties in the compositional analysis and considered two different treatment regimes: "glyphosate treated" (T) and "not treated" (NT): a) For the GM maize stack (T), 63 components in grain and forage were statistically assessed and 39 significantly statistical differences were observed in the Difference Test at 10% significance level. b) For the GM maize stack (NT), 63 components in grain and forage were statistically assessed and 46 significantly statistical differences were observed in the Difference Test at 10% significance level. Following a list of analytes that are statistically significant (as related to the GM maize) in the T + NT treatment regime (FROM CBI: MSL0025987): 1) Proximates and fibre (grain): TDF is significantly lower in both T and NT regime. The relative differences between the test and the control line are -5.7% and -6.8%. Carbohydrates by calculation are significantly higher in both T and NT regime with relative differences of 0.67% and 0.74%. The magnitude of differences of carbohydrates is low (< 1%). The magnitude of differences of TDF is above 5.0% which is a medium level that should be interpreted. It would be helpful if the p-values for TDF were also provided in this context. 2) Proximates and fibre (forage): Calcium is significantly lower in both T and NT regime. The relative differences between the test and the control line are -6.4% and -10.8%. Moisture is significantly lower in both T and NT regime with relative differences of -2.7% and -3.3%. The magnitude of the differences of Calcium is high (> 5-10%) and should be interpreted. It would be helpful if the p-values for TDF were also provided in this context. 3) Total fat and fatty acids: Total fat is significantly lower in both T and NT regime. The relative differences between the test and the control line are -4.9% and -5.6%. Arachidic acid is significantly higher in both T and NT regime with relative differences of 2.7% and 4.1%. Behenic acid is also significantly higher in both	Please see below for considerations on the use of p-values. Please see below for considerations on the use of p-values.

			T and NT regime. The relative differences are medium with 6.6% and 7.4%. Eicosenoic acid is also significantly higher in both T and NT regime with relative differences of 4.9% and 4.7%. The magnitude of the differences of Behenic acid is at a medium level (> 5%) and should be interpreted. It would be helpful if the p-values for this endpoint were also provided and used for interpretation. 4) Protein and amino acids: Protein is significantly lower in both T and NT regime. The relative differences between the test and the control line are -3.3% and -3.8%. 12 amino acids were significantly lower in both T and NT regime and the relative differences range from -2.9% to -8.8%. The NT regime shows higher relative differences, and also two analytes (arginine, cysteine) were classified as "Outcome Type 6, equivalence category (III)", which indicates a statistically significant difference between the Stack and the conventional counterpart, and equivalence to the conventional commercial reference hybrids less likely than not. The magnitude of the differences of the amino acids is at a medium level (> 5%) and should be interpreted. It would be helpful if the p-values were also provided and used for interpretation. 5) Minerals and ash: 5 minerals (of the 8 measured) were significantly lower in both T and NT regime and the relative differences range from -4.5% to -13.7%. The relative differences are -8.8% and -8.9% (iron), -9.5% and -13.7% (manganese), -4.5% and -4.6% (potassium), -10.9% and -12.2% (zinc), and -8.7% and -8.8% (phosphorus). Ash is significantly lower in both T and NT regime. The relative differences between the test and the control line are -7.2% and -4.4%. The magnitude of the differences of the minerals is at a medium to high level (> 4-13%), and of ash at a medium level (> 4-5%) and should be interpreted. It would be helpful if the p-values were also provided and used for interpretation. 6) Vitamins: Vitamin A is significantly lower in both T and NT regime.	Please see below for considerations on the use of p-values. The GMO Panel considered that the changes observed for both treatment regimes in the level of amino acids (measured in % dry weight) were highly correlated with the change in the level of a single analyte, protein in grain; hence, the effective number of significant results was much lower. In order to clarify this point, the GMO Panel requested the applicant to provide a re-analysis of amino acid levels expressed as % amino acids (%AA). In the new analysis (additional information received on 21/4/2017), significant differences were observed only for two and three amino acids in the untreated and treated regime, respectively. In the Scientific Opinion, the GMO Panel reported the results for amino acids expressed as %AA. Please see below for considerations on the use of p-values. Please see below for considerations on the use of p-values.

			The relative differences between the test and the control line are -10.8% and -12.6%. Vitamin B6 is significantly lower in both T and NT regime with relative differences of -7.4% and -9.0%. Vitamin E is also significantly lower in both T and NT regime with relative differences of -7.5% and 5.4%. The magnitude of the differences of the vitamins is at a medium to high level (> 5-12%) and should be interpreted. It would be helpful if the p-values were also provided and used for interpretation.	Please see below for considerations on the use of p-values.
			7) Anti-nutrients: Ferulic acid is significantly lower in both T and NT regime. The relative differences between the test and the control line are -4.5% and -3.6%. Phytic acid is significantly lower in both T and NT regime with high relative differences of -11.7% and -12.0%. Raffinose is also significantly lower in both T and NT regime. The relative differences between the test and the control line are very high with -19.0% and -14.8%. The magnitude of differences of phytic acid and raffinose is high (> 10%) and should be interpreted. It would be helpful if the p-values were provided.	Please see below for considerations on the use of p-values.
			The compositional analysis of the treated (T) regime resulted in 62% significant differences between the GM maize (control line) and the conventional counterpart (test line). The compositional analysis of the not treated (NT) regime resulted in 73% significant differences (i.e. only 17 of 63 endpoints were not significantly different!) between the GM maize (control line) and the conventional counterpart (test line). This high number of significant differences in compositional assessment leads to the conclusion that it is highly likely that the genetic modification of maize stack MON87427xMON89034xMIR162xNK603 resulted in unintended effects.	With the new analysis provided for amino acids levels (see item (4) above), the number of significant differences for the untreated and treated regime was 33 and 28, respectively. The applicant provided an assessment of the number of significant differences using the simulation-based method described in EFSA GMO Panel (2010b). The assessment showed that, when allowance was made for natural background variation, the number of significant results was not a reason of concern.
			The notifier is asked to present a detailed discussion as regards the significances in Difference Tests between the GM maize and the control line (Types 2, 4, 6) with a special regard to analytes that are statistically significant in both treatment regimes.	The GMO Panel took note of this comment.

			The p-values of the difference tests should be submitted and included in the discussion. This can be helpful for further interpretation of the significant differences, in particular in cases where the relative differences between the test and the control lines were high (> 10%). Relative differences are comparisons of means without considering whether the variation within the control population (comparator) is high or low. Thus, it would be more useful to compare both: p-values and relative differences. Potential metabolic shifts should also be considered, since the results at hand indicate a general shift in the metabolism of GM maize MON87427xMON89034xMIR162xNK603 from proteins and fats to the synthesis of carbohydrates. A comparison of the current results and the results from previous notifications on the single events MON87427, MON89034, MIR162, and NK603 would also be a relevant instrument for drawing right conclusions from compositional analysis of the GM maize and its conventional counterpart. In conclusion, there are several points that should be studied and discussed as they are relevant for the risk assessment of the GM maize stack. [MSL0025987 - Dossier EFSA/GMO/NL/2016/131.]	The GMO Panel took note of this comment. In discussing methods for the statistical analysis of field trials (EFSA GMO Panel, 2010b), the GMO Panel argued that – while providing essentially the same information as p-values – confidence intervals give 'a more detailed description of the magnitude of the difference between the GMO and its conventional counterpart'. Therefore, the GMO Panel recommended 'the use of confidence intervals as a standard instrument for the testing of differences as well as equivalence' (EFSA GMO Panel, 2010b). Following the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a), information on confidence intervals was included by the applicant in the compositional analysis report. The potential impact on plant metabolism was among the criteria used by the GMO Panel to assess the significant differences observed between the four-event stack maize and the non-GM comparator. The GMO Panel concluded that none of the differences needed further assessment for food/feed safety (Scientific Opinion, Section 3.4.2). The GMO Panel was able to conclude based on the comparative analysis of the four-event stack maize; a comparison with the results for single events was not needed.

Austria	Fed.Ministry_Health/Women's Aff.	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	1.3.4 Agronomic and phenotypic characteristics: For the assessment of agronomic and phenotypic characteristics material from the field trial discussed under Chapter 1.3.2 was used and analysed. However, relevant information to assess the data provided by the notifier is missing (see comments on the experimental design of the field trials under 1.3.2) and no rationale is provided whether the sample size and the design of the field trial were sufficient to detect potential differences in agronomic and phenotypic characteristics. The notifier fails to provide a rationale for the selection of the phenotypic and agronomic parameters and whether these characteristics are relevant to assess environmental interaction. The assessment falls short of a demonstration that the data basis is sufficient to assess this issue under a range of environmental conditions and that the sample sizes and plot sizes are sufficient to appropriately test such parameters that results would be informative for conditions of commercial agronomic production. The notifier also does not specifically discuss whether the pesticides used for management of the crop adversely affected the assessment of environmental interaction. Therefore, the data submitted for assessment of environmental interaction of GM maize MON87427xMON89034xMIR162xNK603 cannot be adequately evaluated. Thus, the conclusions drawn by the notifier are not sufficiently supported by the submitted data. We request that the notifier provides further information on the abovementioned aspects. Additionally, an analysis of	The experimental design was in line with the applicable EFSA guidance documents (EFSA GMO Panel, 2011a, 2015a). The selected endpoints are representative of the entire life cycle of the crop, from its establishment to harvest, and were considered appropriate according to the applicable guidance documents. Due to the scope of the application, that excludes cultivation, the GMO Panel did not consider necessary to request the additional information suggested by Austria. The genotype-by-enviroment interaction analysis provided by the applicant followed the recommendations of EFSA GMO

			between-site variation should be made to account for interactions of GM maize MON87427xMON89034xMIR162xNK603 with the respective environment (gene x environment interactions). For this analysis the reference range for each site should be calculated from the commercial varieties grown at the specific site. Specific remarks on test design and analysis (FROM CBI: MSL0026080): The results would be more robust and meaningful if multiple-year field trials were performed. Four replications are at least a good standard for field tests. It is unclear if singling was performed. This is likely to be the case, since the plant density at harvest (final stand count) is clearly lower in all trials then after rise (early stand count). Although the yield data are generally acceptable, decisions for omission from statistical analysis of some sites could have been done due to a loss of half (site IARL) or a third (ILRD, INKI) of the plants (e.g. site IARL, block 3, variety Dekalb DKC59-34"…final stand count = 39; site ILRD, block 4, variety "Legacy L7671"…final stand count = 50). Amended report for MSL0026699: phenotypic evaluation and environmental interactions of maize MON 87427 × MON 89034 × MIR162 × NK603 not treated and treated with glyphosate in 2013 U.S. field trials. Dossier EFSA/GMO/NL/2016/131. Amended report for MSL0026699: phenotypic evaluation and environmental interactions of maize MON 87427 × MON 89034 × MIR162 × NK603 not treated and treated with glyphosate in 2013 U.S. field trials - RAW DATA. Dossier EFSA/GMO/NL/2016/131.]	Panel (2010b, 2011a). Per-site summary statistics was also provided to aid the interpretation of the results of the analysis. The GMO Panel was able to conclude based on the information provided by the applicant. The comparative assessment studies followed the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). The GMO Panel considered the field trial design and data analysis adequate for the risk assessment. Data from multiple growing seasons are not among the requirements of the applicable EFSA guidance (EFSA GMO Panel, 2011a). The GMO Panel took note of the comment.
Austria	Fed.Ministry_Health/Women's Aff.	II.1.4.1 Testing of newly expressed proteins	1.4.2 Assessment of newly expressed proteins: For the risk assessment of the newly expressed proteins, a 28-day oral toxicity study in animals is indicated "unless reliable information demonstrating the safety (including mode of action) can be provided, and it is demonstrated that the protein is not structurally and functionally related	The GMO Panel took note of the comment. The proteins newly expressed in GM maize MON 87427 x MON 89034 x MIR162 x NK603 have been previously assessed by the GMO Panel in the context of the single events, and no safety concerns were identified for

			to proteins adversely affecting human or animal health " (EFSA 2011). The Cry1A.105 protein, that is expressed in GM maize MON87427xMON89034xMIR162xNK603, has never been tested via repeated-dose toxicity study but generally argued to be structurally similar (93.6%) to Cry1Ac proteins and functionally (insecticidal activity) similar to Cry1Ab and Cry1F proteins (EFSA 2008). However, the risk assessment of the Cry1Ab and Cry1F proteins dates back to 2005 and is based on "rapid digestion in vitro simulated gastric fluids and for lack of treatment-related toxicity of both proteins in a mouse acute gavage study " (EFSA 2005). The Cry2Ab2, which is expressed in GM maize MON87427xMON89034xMIR162xNK603, also has never been tested via repeated-dose toxicity study. The risk assessment of this Cry protein was performed in 2008 by EFSA and is mainly based on structural identity, "The amino acid sequence of the Cry2Ab2 protein expressed in maize MON89034 is 88% identical to the Cry2Aa protein produced by B. thuringiensis subsp. kurstaki that is frequently used in agriculture for control of insect pests. Use of this bacterial strain has been found safe " (EFSA 2008). Concerning repeated-dose toxicity studies, the applicant remarks, "there is no testable hypothesis to justify the use of experimental animals (EFSA, 2009) to conduct 28-day oral toxicity studies with the CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins. Such testing would not further inform the robust and well established history of safety of these proteins." It is further referred to other notifications, "As demonstrated in details in Sections A.4 of EFSA-GMO-BE-2012-110 and D.8 of EFSA-GMO-NL-2007-37, EFSA-GMO-DE-2010-82 and EFSA-GMO-NL-2005- 22, the CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins and their source organisms have a history of safe consumption. In addition, their modes of action are well established. CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins have no synergistic or antagonistic effects to each other." The notifier's argumentation has the following weaknesses: 1) In order to verify the history of safe consumption of each	humans and animals. The GMO Panel is not aware of any new information that would change this conclusion. In particular, the GMO Panel has assessed the papers quoted by Austria and found these not impacting the previous assessment on these proteins. As regards interactions, the GMO Panel is of the opinion that there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize considering the known biological function of the individual newly expressed proteins. Therefore, no additional studies on these proteins, individually or in combination, are considered necessary by the GMO Panel. Please see Section 3.4.3.3 of the Scientific Opinion for further details.

			newly expressed protein scientifically sound data should be submitted (results of post-market monitoring, epidemiological studies, etc.). This is not the case: Such data were not provided in "Sections A.4 of EFSA-GMO-BE-2012-110 and D.8 of EFSA-GMO-NL-2007-37, EFSA-GMO-DE-2010-82 and EFSA-GMO-NL-2005-22" (to which the notifier refers), and are also not provided in the current Scientific Information (Notification EFSA-GMO-NL-2016-131). The lack of general surveillance and consequently of any exposure data and assessment of already 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. 2) The applicant should perform mode of action tests in appropriate models reflecting mechanisms and processes in human systems for any newly expressed protein with no documented history of safe use (e.g. the chimeric Cry1A.105 protein). 3) The applicant argues that the newly expressed proteins have no synergistic or antagonistic effects to each other. There exists a generalised mechanism of interaction with target organisms (lepidopteran insects) for Cry1A.105 and Cry2Ab2 protein (as outlined in Section D.7.8 of EFSA-GMO-NL-2007-37): a) ingestion of the protoxin by the insect, b) solubilisation in the insect midgut, c) proteolysis of the Cry protein leading to the active delta-endotoxin, d) binding of the endotoxin to receptors of midgut epithelial cells, e) developing of membrane ion channels, f) disruption of cellular homeostasis. The mechanism for the Vip3Aa20 protein is also very similar: The proteolytically processed Vip proteins bind to receptors in the mid-gut epithelium of susceptible insects which follows the formation of membrane ion channels leading to insect death. Even though the mechanisms are very similar, the applicant has not even tested on target organisms the potential for	

			synergistic or antagonistic effects of the Cry proteins and the Vip protein expressed in GM maize MON87427xMON89034xMIR162xNK603, and nonetheless claims that the newly expressed proteins have no synergistic or antagonistic effects to each other. We would like to argue that the applicant has not tested synergistic or antagonistic effects of any of the newly expressed proteins. Furthermore, some feeding studies in mammals have been performed with GM Bt crops and have found adverse effects, such as: • toxic effects or signs of toxicity in the small intestine, liver, kidney, spleen, pancreas, • disturbances in the functioning of the digestive system, • increased or decreased weight gain compared with controls, • male reproductive organ damage, • blood biochemistry disturbances, and • immune system disturbances. As Pardo-López et al. (2009) and Pigott and Ellar (2007) demonstrated, synthetically derived and modified Bt toxins can show higher toxicity than native proteins. Even small changes in the structure of the proteins can cause massive changes in toxicity. Mezzomo et al. (2013) evaluated, in Swiss albino mice, the haematotoxicity and genotoxicity of four Bt spore-crystals genetically modified to express individually Cry proteins administered alone by gavage with a single dose of 27 mg/kg, 136 mg/kg or 270 mg/kg, 24 h, 72 h or 7 days before euthanasia. Their results showed that the Bt spore-crystals genetically modified to express individually Cry proteins can cause some haematological risks to vertebrates, increasing their toxic effects with long-term exposure. Taking into account the increased risk of human and animal exposures to significant levels of these toxins, especially through diet, the authors argue that their results suggest that further studies are required to clarify the mechanism involved in the haematotoxicity found in mice, and to establish the toxicological risks to non-target organisms, especially mammals, before concluding that these microbiological control agents are safe for mammals.	

			Heinemann (2010) stated that "Cry toxin proteins may stimulate an immune response leading to the need to test them as allergens." Finamore et al. (2008) studied gut and peripheral immune responses to genetically modified (GM) maize containing Cry1Ab in mice in vulnerable conditions. The GM maize diet lead to "alterations in the percentage of T and B cells and of CD4+, CD8+, γδT, and αβT subpopulations in mice at the gut and peripheral sites." It was observed that the serum IL-6, IL-13, IL-12p70, and MIP-1beta after MON810 feeding was increased. In conclusion, several questions on the safety of the GM maize and derived food and feed products remain still unanswered and have to be clarified before a final assessment can be made. 28-day oral toxicity studies in rodents could provide the missing information on potential human health effects from exposure to synthetic proteins expressed in the GM maize MON87427xMON89034xMIR162xNK603. [EFSA, 2005. Scientific Opinion of the GMO Panel on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON 863 x MON 810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto. The EFSA Journal 251: 1-22. EFSA, 2008. Scientific Opinion of the GMO Panel on application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto. The EFSA Journal 909: 1-30. EFSA, 2011. Guidance of the GMO Panel for risk assessment of food and feed from genetically modified plants. The EFSA Journal 9(5):2150: 1-37. Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A, Mengheri E, 2008. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. J Agric Food Chem 56(23): 11533-11539.	

			Heinemann JA, 2010. Potential human health risks from Bt plants. Biosafety Briefing, TWN Third World Network, January 2010, 1-10. Mezzomo BP, Miranda-Vilela AL, de Souza Freire I, Barbosa LC, Portilho FA, Lacava ZG, Grisolia CK, 2013. Hematotoxicity of Bacillus thuringiensis as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa in Swiss albino mice. Journal of Hematology & Thromboembolic Diseases 104: doi: 10.4172/2329-8790.1000104. Pardo-López L, Muñoz-Garay C, Porta H, Rodriguez-Almazán C, Soberón M, Bravo A, 2009. Strategies to improve the insecticidal activity of Cry toxins from Bacillus thuringiensis. Peptides 30(3): 589-595. Pigott CR, Ellar DJ, 2007. Role of receptors in Bacillus thuringiensis crystal toxin activity. Microbiol Mol Biol Rev 71(2): 255-281.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.1.4.4 Testing of the whole genetically modified food or feed	1.4.5 Assessment of the whole food and/or feed derived from GM plants: The compositional analysis reveals a high number of significant differences (in the NT regime > 70% of the analytes evaluated) which leads to the conclusion that it is highly likely that the genetic modification resulted in unintended effects. This fact should be given more attention regarding the toxicological assessment of the whole GM maize MON87427xMON89034xMIR162xNK603. Nevertheless, the notifier has not considered it necessary to carry out a 90-day toxicity study in rodents with the GM maize; and thus, no final evidence is possible with reference (even) to sub-chronic effects of the whole food and feed. Moreover, potential long-term (especially appropriate for foodstuffs), reproductive or developmental effects of the whole food and/or feed are not assessed by the notifier.	The GMO Panel took note of the comment. Based on the outcome of the molecular characterisation assessment, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety have been identified related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern have been identified in the composition of the four-event stack maize. Therefore, in line with EFSA GMO Panel (2011a) and Regulation (EU) 503/2013 animal studies on food/feed derived from the four-event stack maize are not necessary.
Austria	Fed.Ministry_Health/Women's Aff.	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	Scientific Information, Chapter 5.3.1.2. Step 2: Hazard characterization: The risk assessment rationale presented by the applicant is largely based on the foreseen use of the plants, which excludes cultivation and thus focuses on the assumption that only single plants emerge under unfavourable	The GMO Panel considers unlikely that the intended traits of event MON 87427 × MON 89034 × MIR162 × NK603 will provide a selective advantage to maize plants, except when they are exposed to glyphosate-containing herbicides or infested by

			conditions. Under certain circumstances (seed dispersal, mild winters, cultivation practice, etc.), volunteers may occur in the fields and not be eliminated by cultivation and plant protection measures. In his characterisation, the applicant has not taken this type of event into consideration. The insect tolerance traits would furthermore confer a selective advantage independent of cultivation practice, like the application of pesticides.	insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins. However, the GMO Panel considers this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors limiting plant's persistence and invasiveness. Therefore, the presence of the intended traits will not affect the persistence and invasiveness of the GM plant.
Austria	Fed.Ministry_Health/Women's Aff.	II.5.3.2 Plant to micro-organisms gene transfer	Scientific Information, p. 86: The applicant maintains that "there is negligible potential for recombination between genetic material inherited in GM maize MON87427xMON89034xMIR162xNK603 and environmental prokaryotic micro-organisms due to limited bacterially derived sequence content […] and the absolute requirement of the presence of a homologous sequence in the acceptor prokaryotic micro-organism." We would like to indicate that 66% (= 3.2 kb) of the transgenic insert of MON87427 and 40% (= 9.56 kb) of the transgenic insert of MON89034xNK603 are bacterially derived sequences (according to Tables 2 and 3, Part II/Scientific Information - EFSA-GMO-BE-2013-117). This is a significant proportion of microbial DNA in the DNA element transferred into the plant genome. In relation to the total genomic DNA of transgenic plants the bacterial sequences are indeed only a minute fraction, however, we would like to point to the fact that transgenic insert DNA is detectable between two to four years in soil environments and, thus, available for natural transformation and recombination with the genome of competent bacteria (Gebhard and Smalla 1999; Pontiroli et al. 2010). The applicant maintains that "if the introduction of cp4 epsps, cry1A.105, cry2Ab2, Vip3Aa20 and pmi genes does offer an evolutionary advantage, the genes would have been transferred to other microbes during evolution via HGT from microbes already possessing this gene." This is a naïve perception of evolutionary processes active in naturally occurring bacterial populations. Even the multitude of existing cry gene variants (Crickmore et al. 1998; Patel et al. 2013) is indicative for a highly recombinogenic nature of the involved gene elements (de Maagd et al. 2001) and for a genetic locus under severe selection pressure providing substantial and selective "evolutionary advantage" to cry gene variant carriers.	The GMO Panel took note of the comment. The issues identified by Austria are addressed below.

			There is a multitude of B. thuringiensis strains each carrying certain variants of cry genes (Bravo et al. 2013) which presumably have evolved inter alia via the exchange of gene fragments (i.e. by HGT) (de Maagd et al. 2001). The applicant concludes that "therefore the risk of HGT is negligible from GM plant to a micro-organism since cp4 epsps, cry1A.105, cry2Ab2, Vip3Aa20 and pmi genes are already present in microbial populations. Owing to the natural occurrence of cp4 epsps, cry1A.105, cry2Ab2, vip3aa20 and pmi genes in the environment, a low-level gene transfer to the bacteria is thought not to confer a new trait and selective advantage." The presented risk assessment of a potential plant-to-bacteria gene transfer is suffering from the following issues: 1) The applicant does not take into account partial DNA transfers of fragments of the transgenic insert coding for prokaryotic elements. 2) The applicant does not take into account the formation of mosaic genes potentially created by transgenic insert derived DNA fragments (Woegerbauer et al. 2015). 3) The applicant does not take into account homology-directed illegitimate recombination as relevant process for generating genetic diversity under involvement of transgenic insert derived DNA fragments (de Vries and Wackernagel 2002). 4) The applicant does not take into account that the transgenic insert is affected by the same plant-intrinsic mutation rate as any plant gene and may contain single nucleotide polymorphisms after release from the plant cells and may thus provide genetic variability upon recombination with similar bacterial sequences leading to alterations in the substrate specificity of EPSPS which may in turn result into changes in the population structure of	1) HGT considers the transfer of genetic elements which would be functional in recipients and provide a selective advantage. The transfer of DNA fragments could become relevant if connected to a selective advantage; however, in the assessment of the four-event stack maize, no hypothesis was found that this could be the case (see Scientific Opinion, Section 3.3.4.2). 2) The potential formation of mosaic genes is taken into account in the HGT assessment (see Scientific Opinion, Section 3.3.4.2). 3) 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. In addition, because of plant codon optimisation, the recombinant gene products are probably less functional than their natural variants in bacterial cells 4 and 5) There is no indication that point mutations in the transgenic DNA would be of concern. Recombination with bacteria and related environmental consequences were considered (see replies to previous comments on HGT).

			exposed bacterial communities. 5) The applicant does not take into account that plant-derived DNA may suffer lesions (e.g. abasic sites, etc.) in natural environments (soil, gastrointestinal tract) which may act mutagenic upon successful recombination in the bacterial host generating genetic variability of the affected host gene (Overballe-Petersen et al. 2013). 6) The applicant does not acknowledge that low gene transfer rates are not informative for long-term effects on bacterial populations relevant for human, animal or environmental health (Pettersen et al. 2005). 7) The applicant generally assumes an absence of selection pressure which would be necessary for the fixation of a new trait in the exposed bacterial population and ignores the fact that at present mechanisms responsible for exerting selection pressure on bacterial populations in natural habitats are not well understood and characterised. Considering the inconsistencies present in the risk assessment of plant-to-bacteria gene transfer processes as summarised above (see points 1-7), we are of the opinion that the conclusions as drawn by the applicant are affected by a certain number of uncertainties which compromise the risk assessment substantially. [Bravo A, Gómez I, Porta H, García-Gómez BI, Rodriguez-Almazan C, Pardo L, Soberón M, 2013. Evolution of Bacillus thuringiensis Cry toxins insecticidal activity. Microbial biotechnology 6(1): 17-26. Crickmore N, Zeigler DR, Feitelson J, Schnepf E, Van Rie J, Lereclus D, Baum J, Dean DH, 1998. Revision of the nomenclature for the Bacillus thuringiensis pesticidal crystal proteins. Microbiol Mol Biol Rev 62(3): 807-813. de Maagd RA, Bravo A, Crickmore N, 2001. How Bacillus thuringiensis has evolved specific toxins to colonize the insect world. Trends Genet 17(4): 193-199. de Vries J, Wackernagel W, 2002. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. PNAS 99(4): 2094-2099.	6) Long-term effects are addressed by general post-market monitoring. There are no HGT-related issues/indications which would justify a casespecific post-market monitoring 7) Any remaining uncertainty connected to environmental risks would have to be addressed by the post-market environmental monitoring. However, based on current knowledge, no such uncertainty was identified in the assessment of the four-event stack maize.

			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. Overballe-Petersen S, Harms K, Orlando LA, Mayar JV, Rasmussen S, Dahl TW, Rosing MT, Poole AM, Sicheritz-Ponten T, Brunak S, Inselmann S, de Vries J, Wackernagel W, Pybus OG, Nielsen R, Johnsen PJ, Nielsen KM, Willerslev E, 2013. Bacterial natural transformation by highly fragmented and damaged DNA. Proc Natl Acad Sci U S A 110(49):19860-19865 Patel KD, Purani S, Ingle SS, 2013. Distribution and diversity analysis of Bacillus thuringiensis cry genes in different soil types and geographical regions of India. J Invertebr Pathol 112(2): 116-121. Pettersen AK, Bohn T, Primicerio R, Shorten PR, Soboleva TK, Nielsen KM, 2005. Modeling suggests frequency estimates are not informative for predicting the long-term effect of horizontal gene transfer in bacteria. Environ Biosafety Res 4(4): 223-233. Pontiroli A, Ceccherini MT, Pote J, Wildi W, Kay E, Nannipieri P, Vogel TM, Simonet P, Monier JM, 2010. Long-term persistence and bacterial transformation potential of transplastomic plant DNA in soil. Res Microbiol 161(5): 326-334. Woegerbauer M, Kuffner M, Domingues S, Nielsen KM, 2015. Involvement of aph(3')-IIa in the formation of mosaic aminoglycoside resistance genes in natural environments. Frontiers in Microbiology 6.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.6 Post-Market Environmental Monitoring Plan (PMEM)	6.1 General: The proposed monitoring plan cannot be considered adequate for the following reasons: The notifier does not specifically consider potential exposure of EU environments to GM maize MON87427xMON89034xMIR162xNK603 other than by unintended release of substantial volumes of GM maize MON87427xMON89034xMIR162xNK603 e.g. via losses during loading or unloading for processing into animal feed or human food products. Other exposure scenarios should	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 environmental risk assessment (ERA) of the four-event stack maize is mainly concerned with: (1) the exposure of bacteria to recombinant DNA in the gastrointestinal tract of animals fed GM material and bacteria present in environments exposed to faecal material of these animals (manure and faeces); and (2) the accidental release into the environment of viable maize

			be considered according to current EFSA guidance (EFSA 2011), e.g. accidental spillage during transport, commingling with other maize grain lots and exposure via waste materials from processing or use. Since all exposure pathways should be taken into account in the monitoring plan appropriately, we consider the monitoring plan at hands to be insufficient to address the potential environmental effects of GM maize MON87427xMON89034xMIR162xNK603. [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.]	MON 87427 x MON 89034 x MIR162 x NK603 grains during transportation and/or processing. Moreover, 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.
Austria	Fed.Ministry_Health/Women's Aff.	II.6.2 Case Specific Monitoring (strategy, method and analysis)	6.2.1 Case-specific GM plant monitoring: The notifier does not present a plan for monitoring the environmental exposure by GM maize MON87427xMON89034xMIR162xNK603 using appropriate methods (i.e. standardised methodologies for sampling and identification of GM maize MON87427xMON89034xMIR162xNK603). Since the ERA presented for GM maize MON87427xMON89034xMIR162xNK603 in our opinion is associated with uncertainties, Case Specific Monitoring (CSM) should be implemented to address the respective issues. Specifically, the extent of exposure of the environment to GM maize MON87427xMON89034xMIR162xNK603, the fate of transgenic materials in the environment and potential environmental impacts should be addressed by CSM (compare Züghart et al. 2011). The general recommendations by EFSA from the evaluation of previous monitoring of other GM crops (among others EFSA 2011; EFSA 2012) should be considered by the notifier and appropriate suggestions (e.g. as regards the literature review, etc.) implemented. [EFSA, 2011. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON810 in 2009. The EFSA Journal 9(10):2376: 1-66.	As the environmental risk assessment did not identify potential adverse environmental effects from the four-event stack maize, the GMO Panel did not require case-specific monitoring.

			EFSA, 2012. Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. The EFSA Journal 10(4):2610: 1-35. Züghart W, Raps A, Wust-Saucy A-G, Dolezel M, Eckerstorfer M, 2011. Monitoring of genetically modified organisms. A policy paper representing the view of the National Environment Agencies in Austria and Switzerland and the Federal Agency for Nature Conservation in Germany. Umweltbundesamt Reports 305. Vienna: 1-56.]	
Austria	Fed.Ministry_Health/Women's Aff.	II.6.3 General Surveillance (strategy, method)	6.2.2 General surveillance for unanticipated adverse effects: As noted in the general comment all routes of exposure of the environment should be taken into account in GS, including exposure to (waste) materials from processing or use. The requirement that all potential routes of exposure should be addressed by the proposed monitoring is one of the pillars of the EU-approach to monitoring and included in the current EFSA guidance for PMEM (EFSA 2011). The description of the monitoring methodology does not exactly indicate which specific information will be gathered by General Surveillance (GS). The notifier thus should describe in more detail the monitoring methodology and which data are gathered by GS and how. The notifier only states that the responsibilities for the General Surveillance of GM maize MON87427xMON89034xMIR162xNK603 are shared between the authorisation holder and third parties, such as operators involved in the import, handling and processing of viable GM maize MON87427xMON89034xMIR162xNK603 (e.g. traders, silo operators, processors). These operators, represented by trade associations and existing networks (e.g. COCERAL, UNISTOCK, FEDIOL), are obliged to report any potential unanticipated adverse effect to the authorisation holder. However, these organisations and companies are not specified in detail by the notifier. Thus it remains unclear who will conduct the monitoring in practice. It is therefore not possible to evaluate the efficacy of the monitoring, which will be influenced by the availability, extent and	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.

			composition of existing networks in EU Member States as well as their commitment as regards the monitoring goals. The notifier should therefore indicate the national organisations which will be involved in each individual EU Member State and provide statements indicating their willingness to participate. It must be clear before placing on the market of GM maize MON87427xMON89034xMIR162xNK603 which existing networks will be involved and to which degree they will be involved. Furthermore, the notifier has not selected other networks further down the food/feed production chain for General Surveillance. However, environmental effects of food/feed processing and the use of GM maize MON87427xMON89034xMIR162xNK603 in food or feed must be taken into account according to Regulation (EC) 1829/2003 (Art. 5.5b and Art.17.5b). Therefore e.g. respective medical or veterinary networks should be involved for the surveillance of unanticipated effects on human and animal health. The methodology of the proposed General Surveillance is based on passively collecting information. A proactive approach of GS, including specific activities for monitoring of accidental spillage and the potential establishment of GM maize MON87427xMON89034xMIR162xNK603 in the environment, should also be proposed and implemented by the notifier (see general remarks to this notification). The notifier states that the surveillance is based on HACCP principles without giving details on the specific approach. Thus it is unclear how these principles match with the requirements of environmental monitoring of GM maize MON87427xMON89034xMIR162xNK603. The general reference to HACCP principles as included in the monitoring plan thus needs to be better specified by the notifier. In conclusion, the proposed monitoring plan is considered inappropriate for addressing relevant issues of PMEM of GM maize and thus cannot be regarded as sufficiently elaborated for the monitoring of potential environmental exposure by GM maize	

			MON87427xMON89034xMIR162xNK603. [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.]	
Belgium	Biosafety Advisory Council	II.1.2.2 Information relating to the genetically modified plant	Why using MON87427 and NK603 in the stacked event (MON87427 x MON89034 x MIR162 x NK603), since both provide tolerance to glyphosate? Is the reason to have a higher level of EPSPS? And why is this needed? See table 2 p 27: EPSPS levels, both in forage and grain in the stacked event are almost double the EPSPS levels in MON87427 or NK603 separately.	The presence of two copies of the gene expressing EPSPS resulted in nearly two-fold level of the protein in the stack compared to the respective singles carrying only one copy of the gene. The GMO panel considers that the EPSPS protein level in the stack simply reflects the presence of two copies of the EPSPS gene and is not an indication of interaction between the events in the stack.
Belgium	Biosafety Advisory Council	II.1.2.2 Information relating to the genetically modified plant	In Table 5 and 6 on pgs. 30 and 31 respectively, the data describing the expression levels of the proteins on a fresh weight basis is missing, while this is included in Tables 3 & 4. What is the reason?	The GMO Panel acknowledges that the data describing the expression levels of the proteins on a fresh weight basis is indeed absent in Tables 5 and 6 of the technical dossier. However, it is the expression levels determined on the basis of dry weight reported for all tissues (from all plants tested) that were used in the risk assessment. The GMO Panel considers that the protein expression data provided by the applicant is sufficient to conclude on the risk assessment.
France	DGCCRF - Ministère Consommation	Part II – Scientific information	Conclusions du Groupe de travail « Biotechnologie » de l'Anses En l'absence de données susceptibles de lever les réserves précédemment exprimées au sujet du maïs MON89034, le GT « Biotechnologie » émet un avis défavorable à la demande d'autorisation de mise sur le marché, au titre du règlement (CE) n° 1829/2003, du maïs MIR162 x MON87427 x MON89034 x NK603 et des dix sous-combinaisons contenant deux ou trois des événements MIR162, MON87427, MON89034 et NK603. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE L'Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail endosse les conclusions du Groupe de travail « Biotechnologie » et émet un avis défavorable à la demande d'autorisation de mise sur le marché, au titre du règlement (CE) n° 1829/2003, du maïs MIR162 x MON87427 x MON89034 x NK603 et des dix sous-combinaisons contenant deux ou trois des événements MIR162, MON87427, MON89034 et NK603. **ENGLISH TRANSLATION**	

			In the absence of data capable of removing the reservations previously expressed with regard to maize MON89034, the 'Biotechnology' Working Group issues an unfavourable opinion with regard to the application for a marketing authorisation, pursuant to Regulation (EC) No 1829/2003, for maize MIR162 x MON87427 x MON89034 x NK603 and for the ten sub-combinations containing two or three of the events MIR162, MON87427, MON89034 and NK603. CONCLUSIONS AND RECOMMENDATIONS OF THE AGENCY The *Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail* (Anses) [French Agency for Food, Environmental and Occupational Health and Safety] endorses the conclusions of the 'Biotechnology' Working Group and issues an unfavourable opinion with regard to the application for a marketing authorisation, pursuant to Regulation (EC) No 1829/2003, for maize MIR162 x MON87427 x MON89034 x NK603 and for the ten sub-combinations containing two or three of the events MIR162, MON87427, MON89034 and NK603.	
France	DGCCRF - Ministère Consommation	Part II – Scientific information	Evaluations antérieures et autorisations de mise sur le marché Les quatre maïs parentaux et deux sous-combinaisons du maïs MIR162 x MON87427 x MON89034 x NK603 ont été évalués par l'Afssa ou l'Anses, dans le cadre d'une demande d'autorisation de mise sur le marché au titre du Règlement (CE) n° 258/97, de la Directive 2001/18/CE ou du Règlement (CE) n° 1829/2003. Concernant les maïs MIR162, MON87427 et NK603, l'Agence a conclu que la consommation de ces maïs et de leurs dérivés présente le même niveau de sécurité sanitaire pour l'Homme et l'animal que la consommation de maïs non génétiquement modifiés et de leurs dérivés. En revanche, les avis de l'Agence relatifs à la demande d'autorisation de mise sur le marché du maïs MON89034 sont défavorables. En effet, dans son avis du 20 novembre 2007, l'Afssa avait demandé que des explications complémentaires soient apportées sur la différence d'apparition des calculs dans la vessie entre les données historiques (0,49 %) et l'incidence de 10 % (base 20 animaux) observée chez les animaux femelles du groupe ayant ingéré la forte dose de	

			MON89034 (Afssa, 2007a). Bien que des données historiques provenant de 70 études conduites entre 1999 et 2006 avec des rats de la même souche aient été fournies par le pétitionnaire, elles ne sont pas apparues suffisantes pour permettre de conclure à l'absence de lien entre l'administration orale de maïs MON89034 et la survenue des calculs de la vessie observés chez les animaux femelles nourris à la forte dose de MON89034 (Anses, 2012). Les avis de l'Agence concernant les demandes d'autorisation de mise sur le marché des maïs MON89034 x NK603 (Afssa, 2007b et 2010) et MON87427 x MON89034 x NK603 (Anses, 2015) sont également défavorables, en l'absence d'explications convaincantes sur l'origine de l'incidence des calculs vésicaux soulevée lors de l'examen du maïs MON89034 ou d'études de toxicité sub-chronique de 90 jours sur rongeur réalisées avec ces maïs. ---------- Afssa (2007a). Avis de l'Agence française de sécurité sanitaire des aliments du 20 novembre 2007 relatif à un dossier d'autorisation de mise sur le marché d'un maïs génétiquement modifié MON 89034 résistant à des insectes, pour l'importation et l'utilisation en alimentation humaine et animale de grains et de ses produits dérivés, au titre du règlement (CE) n° 1829/2003. Afssa (2007b). Avis de l'Agence française de sécurité sanitaire des aliments du 20 novembre 2007 relatif à un dossier d'autorisation de mise sur le marché d'un maïs génétiquement modifié MON 89034 x NK 603 résistant à des insectes et tolérant à un herbicide, pour l'importation et l'utilisation en alimentation humaine et animale de grains et de ses produits dérivés, au titre du règlement (CE) n° 1829/2003. Afssa (2010). Avis de l'Agence française de sécurité sanitaire des aliments du 29 janvier 2010 relatif à un dossier d'autorisation de mise sur le marché du maïs hybride génétiquement modifié MON 89034 x NK 603, tolérant à un herbicide et résistant à des insectes, pour la culture, l'importation et la transformation ainsi que l'utilisation en alimentation humaine et animale de cet OGM, au titre du règlement (CE) n° 1829/2003. Anses (2012). Avis de l'Agence nationale de sécurité	

			sanitaire de l'alimentation, de l'environnement et du travail du 25 juillet 2012 relatif à un dossier de demande de mise sur le marché, au titre du règlement (CE) n° 1829/2003, du maïs génétiquement modifié MON89034, résistant à certains insectes, pour la culture. Anses (2015). Avis de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail du 1er septembre 2015 relatif à une demande d'autorisation de mise sur le marché, au titre du Règlement (CE) n° 1829/2003 relatif aux denrées et aux aliments pour animaux génétiquement modifiés, du maïs génétiquement modifié MON87427 x MON89034 x NK603, développé pour être résistant à certains insectes et tolérant au glyphosate, pour l'importation, la transformation, ainsi que l'utilisation en alimentation humaine et animale de cet OGM (dossier n° EFSA-GMO-BE-2013-117). ENGLISH TRANSLATION Previous assessments and marketing authorisations The four maize parents and two sub-combinations of maize MIR162 x MON87427 x MON89034 x NK603 were assessed by Afssa [*Agence française de sécurité sanitaire des aliments* – French Food Safety Agency] or Anses [*Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail* – French Agency for Food, Environmental and Occupational Health and Safety], in the context of an application for a marketing authorisation pursuant to Regulation (EC) No 258/97, Directive 2001/18/EC or Regulation (EC) No 1829/2003. The Agency concluded that the consumption of maize MIR162, MON87427 and NK603, and derivatives thereof, presents the same level of safety for human or animal health as the consumption of non-genetically modified maize and derivatives thereof. However, the Agency's opinions relating to the application for a marketing authorisation for maize MON89034 are unfavourable. In its opinion of 20 November 2007, Afssa requested that further explanations be provided on the difference in development of bladder stones between historical data	The GMO Panel took note of the comments. In accordance to Regulation (EU) No 503/2013, in the context of the current application on the four-event stack maize the applicant provided a 90-day oral repeated-dose toxicity study in rats on whole food and feed from each of the maize single-event MON 87427, MON 89034, MIR162 and NK603. The four studies had already been provided in the context of the single-event applications and assessed by the GMO Panel; no adverse effects related to the administration of the respective GM diets had been identified. As regards specifically the 90-day study on MON 89034, the GMO Panel thoroughly assessed it in the context of application EFSA-GMO-NL-2007-37 (EFSA, 2008). The numerically higher incidence of kidney alterations observed in females of the high dose group was attributable to two rats (one died at day 14 of unknown cause, the other survived to the end of the study). The alterations in these two rats included minimal chronic progressive nephropathy, minimal/moderate transitional cell hyperplasia, minimal sub-acute inflammation and moderate hydronephrosis. The animal that died on day 14 additionally showed mild papillary necrosis and minimal tubular necrosis. Both rats had urinary bladder calculi and the study pathologist concluded that the lesions observed most likely were linked to

			(0.49%) and the 10% incidence (based on 20 animals) seen in female animals in the group which had ingested a high dose of MON89034 (Afssa, 2007a). Although the historical data from 70 studies conducted between 1999 and 2006 with rats from the same strain were submitted, they did not appear sufficient to justify a conclusion that there was no link between oral administration of maize MON89034 and development of bladder stones in female animals fed with the high dose of MON89034 (Anses, 2012). The Agency's opinions regarding the applications for a marketing authorisation for maize MON89034 x NK603 (Afssa, 2007b and 2010) and MON87427 x MON89034 x NK603 (Anses, 2015) are also unfavourable, in the absence of convincing explanations as to the origin of the incidence of bladder stones raised during the examination of maize MON89034 or of 90-day sub-chronic toxicity studies on rodents carried out with these types of maize. Afssa (2007a). Opinion of the French Food Safety Agency of 20 November 2007 on the marketing authorisation file for genetically modified maize MON89034 resistant to insects, for importation and use of the grain and derivatives thereof in human and animal foodstuffs, pursuant to Regulation (EC) No 1829/2003. Afssa (2007b). Opinion of the French Food Safety Agency of 20 November 2007 on the marketing authorisation file for genetically modified maize MON 89034 x NK 603, resistant to insects and tolerant to a herbicide, for importation and use of the grain and derivatives thereof in human and animal foodstuffs, pursuant to Regulation (EC) No 1829/2003. Afssa (2010). Opinion of the French Food Safety Agency of 29 January 2010 on the marketing authorisation file for genetically modified maize MON 89034 x NK 603, tolerant to a herbicide and resistant to insects, for cultivation, importation, processing and also use of this GMO in human and animal foodstuffs, pursuant to Regulation (EC) No 1829/2003.	these calculi. It seems unlikely that the urinary bladder calculi and associated kidney alterations could have been induced by the tested maize in 14 days. A low incidence of urinary bladder calculi is known to occur in this rat strain and may be considered a spontaneous finding in sub-chronic studies. According to historical control data supplied in the application, the incidence of urinary bladder calculi in high dose females in this study was also found in female control rats in previous studies conducted with CD rats in the same testing laboratory. The GMO Panel therefore considered the urinary bladder calculi as well as the associated kidney alterations as incidental findings which were not related to administration of maize MON 89034. The same applied to the nephroblastomas, a very rare tumour of the kidney, which were observed in two female animals of the control group (EFSA, 2008). In the context of the assessment of this four-event stack maize and in order to fulfil the requirements of Regulation (EU) No 503/2013 for 90-day studies, the applicant provided additional information upon EFSA's request: missing information on test material and diets for all the studies; evaluation of the cage effect in the study on MIR162; and additional histopathological analysis for the studies on MON 87427 and MON 89034. The additional information provided allowed the GMO Panel to conclude that these studies are aligned with regulation(EU) 503/2013 and to confirm its previous conclusions, i.e. that there are no indications of adverse effects related to the 90-day administration to rats of diets including grains from maize MON 87427 (33%), MON 89034 (up to 33%) MIR162 (up to 41%) and NK603 (up to 33%).

			Anses (2012). Opinion of the French Agency for Food, Environmental and Occupational Health and Safety of 25 July 2012 on a marketing authorisation file, pursuant to Regulation (EC) No 1829/2003, for genetically modified maize MON89034, resistant to certain insects, for cultivation. Anses (2015). Opinion of the French Agency for Food, Environmental and Occupational Health and Safety of 1 September 2015 on an application for a marketing authorisation, pursuant to Regulation (EC) No 1829/2003 relating to genetically modified foodstuffs and animal feed, for genetically modified maize MON87427 x MON89034 x NK603, developed to be resistant to certain insects and tolerant to glyphosate, for importation, processing and also use of this GMO in human and animal foodstuffs (file No EFSA-GMO-BE-2013-117).	
Germany	BfN	II.1 Hazard identification and characterisation	The Federal Agency for Nature Conservation (BfN) considers that further information should be presented before the risk assessment of EFSA/GMO/NL/2016/131 can be finalised. Agronomic data should be amended and include data on the occurrence of volunteers. Although MON87427xMON89034xMIR162xNK603 maize is not intended for cultivation we strongly suggest that the applicant provides detailed information on the wild relative teosinte, which has been reported in EU fields. As spillage of maize seed during transport is possible, the introgression of the Bt trait into teosinte has to be considered. Insect-resistance, however, may increase fitness in teosinte and thus the likelihood of the plant to become invasive or a pest problem (Hjältén et al. 2012, Letourneau et al. 2003, 2012; Meier et al. 2013; Snow et al. 2003, Steward et al. 1997; Vacher et al. 2004; Wilkinson & Tepfer 2009; Yang et al. 2012). Hjältén, J., Axelsson, E. P., Whitham, T. G., LeRoy, C. J., Julkunen-Tiitto, R., Wennström, A. & Pilate, G. (2012) Increased resistance of Bt aspens to Phratora vitellinae (Coleoptera) leads to increased plant growth under experimental conditions. PLoS ONE, 7, 30640. Letourneau, D. K. & Hagen, J. A. (2012) Plant fitness assessment for wild relatives of insect resistant Bt-crops. Journal of Botany, 2012, 389247; DOI 10.1155/2012/389247.	Information on maize volunteers is not required in the comparative analysis of agronomic and phenotypic characteristics. Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.

			Letourneau, D. K., Robinson, G. S. & Hagen, J. A. (2003) Bt crops: Predicting effects of escaped transgenes on the fitness of wild plants and their herbivores. Environmental Biosafety Research, 2 (4), 219-246. Meier, M. S., Trtikova, M., Suter, M., Edwards, P. J. & Hilbeck, A. (2013) Simulating evolutionary responses of an introgressed insect resistance trait for ecological effect assessment of transgene flow: a model for supporting informed decisionmaking in environmental risk assessment. Ecology and Evolution, 3, 416-432. Snow, A. A., Pilson, D., Rieseberg, L. H., Paulsen, M. J., Pleskac, N., Reagon, M. R., Wolf, D. E. & Selbo, S. M. (2003) A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecological Applications, 13 (2), 279-286. Steward, C. N., All, J. N., Raymer, P. L. & Ramachandran, S. (1997) Increased fitness of transgenic insecticidal rapeseed under insect selection pressure. Molecular Biology, 6, 773-779. Vacher, C., Weis, A. E., Hermann, D., Kossler, T., Young, C. & Hochberg, M. E. (2004) Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (Brassica rapa). Theoretical and Applied Genetics, 109, 806-814. Wilkinson, M. & Tepfer, M. (2009) Fitness and beyond: Preparing for the arrival of GM crops with ecologically important novel characters. Environmental Biosafety Research, 8, 1-14. Yang, X., Wang, F., Su, J. & Lu, B. (2012) Limited fitness advantages of crop-weed hybrid progeny containing insect-resistant transgenes (Bt/CpTI) in transgenic rice field. PLoS ONE, 7, 41220.	
Germany	BfN	II.1.3.1 Choice of the conventional counterpart and additional comparators	The choice of comparators is not appropriate to assess possible combinatorial effects in the stacked event (potential interactions between the events). Therefore, we suggest including the GM parental materials, i.e. the single events, as well as appropriate non-transgenic genotype(s) in the comparative analyses. Plant material used for comparative assessment including the GMO and the comparator were tested for their purity. However, neither test conditions nor results are presented. Tests for absence of other GM events in the control and reference lines are missing. The applicant is asked to provide tests demonstrating the	The experimental design was in line with the applicable EFSA guidance documents (EFSA GMO Panel, 2011a, 2015a), where the inclusion of the GM parental materials is not requested. Possible combinatorial effects are directly quantified in the protein expression analysis where data for the parental lines are required. Following a request of the GMO Panel, the applicant provided further information to characterise the quality of the starting materials (additional information received on 29/9/2016).

			purity of the control and comparator starting seed (cf. EFSA's request from 06.06.2016 in case of application EFSA-126 to provide purity levels for analysed seeds).	
Germany	BfN	II.1.3.4 Comparative analysis of composition	To demonstrate effects of the depth of intervention in the 4-stacked event MON87427xMON89034¬xMIR162¬xNK603 maize and in order to identify potential unintended interactions, results of the compositional analysis were assessed in comparison with the 3-stacked event MON87427xMON89034xNK603 (EFSA-117) and the single event MON87427 (EFSA-110). Other related events, i.e. single events MON89034 and NK603 and the stacked event NK603xMON89034, were not considered, because compositional analysis of those events didn't include an outcome type classification of the analytes. Results of the difference test demonstrated minor differences between the GMO and the non-modified counterpart in the single event (13 %) and increasing differences for the stacked events (78 % and 81 % for the 3-stacked and 4-stacked events, respectively). Results of the equivalence test demonstrated an increasing number of analytes exceeding the range of natural variance with increased stacking (category II: 2 %, 9 % and 14 %; category III: 0 %, 4 % and 6 % for MON87427, MON87427xMON89034xNK603 and MON87427xMON89034xMIR162xNK603, respectively). The following conclusions can be drawn: a) Differences in the majority of plant metabolites in MON87427xMON89034xMIR162xNK603 maize compared to the non-modified counterpart (which were not visible in the single event MON87427) indicate systemic alterations in plant metabolism. This may be caused by unintended interactions in the stacked event. According to EFSA (2011) "the test of difference is used to verify 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", we conclude that MON87427xMON89034xMIR162xNK603 maize has the potential to cause adverse effects. b) About 20 % of the analytes in grain (mainly amino acids) exceeded the range of natural variance in MON87427xMON89034xMIR162xNK603 maize, indicating that substantial parts of plant metabolism exceed natural variance and hence unpredictable effects in terms of	The GMO Panel took note of the comment. The GMO Panel considered that the changes observed for both treatment regimes in the level of amino acids (measured in % dry weight) were highly correlated with the change in the level of a single analyte, protein in grain; hence, the effective number of significant results was much lower. In order to clarify this point, the GMO Panel requested the applicant to provide a re-analysis of amino acid levels expressed as % amino acids (%AA). In the new analysis (additional information received on 21/4/2017), significant differences were observed only for two and three amino acids in the untreated and treated regime, respectively. In the Scientific Opinion, the GMO Panel reported the results for amino acids expressed as %AA. a) The potential impact on plant metabolism was among the criteria used by the GMO Panel to assess the significant differences in forage and grain composition observed between the four-event stack maize and the non-GM comparator. The GMO Panel concluded that none of the differences needed further assessment for food/feed safety (Scientific Opinion, Section 3.4.2). b) In the re-analysis provided by the applicant (additional information received on 21/4/2017), the level of all amino acids (expressed as %AA) fell into equivalence category I or II, except for serine levels which were not categorised for

			environmental behaviour or synthesis of metabolites relevant for food and feed safety need to be considered in detail in the ERA. In conclusion there is evidence that the genetic modifications cause unintended effects in the stacked event MON87427xMON89034xMIR162xNK603 maize, resulting in alterations of plant metabolism beyond natural variance. In order to assess whether those alterations pose a risk in terms of environmental or food and feed safety it is crucial i) to detect the reason for these systemic alterations and ii) to identify metabolic pathways and functions, which are affected by those. EFSA Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011 9(5):2150	equivalence. In the Scientific Opinion, the GMO Panel reported the results for amino acids expressed as %AA. The GMO Panel assessed all significant differences between the four-event stack maize and the non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. The GMO Panel concluded that none of the differences needed further assessment for food/feed safety (Scientific Opinion, Section 3.4.2).
Germany	BfN	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	The current application has the scope for import and processing; the applicant provided phenological and agronomic data from eight US field locations in 2013. For the import of viable maize seed the main concern during import and processing would be that the GMO could survive in the environment after loss and spillage. Survivability could be enhanced due to expression of Bt proteins (see II.5.3.1) and alterations of metabolic pathways (see II.1.3.4). We recommend, to include data on the occurrence of volunteers after the field trials. Palaudelmàs et al. (2009) have found maize volunteers on a regular basis in Spain. Also, the recommendation of the EU Commission – amongst others – to remove volunteer maize plants in order to control Diabrotica in the EU indicates that overwintering of maize under EU conditions is to be expected. Palaudelmàs, M., Peñas, G., Melé, E., Serra, J., Salvia, J., Pla, M., Nadal, A., Messeguer, J. (2009). Effect of volunteers on maize gene flow. Transgenic Res 18: 583–594. DOI 10.1007/s11248-009-9250-7. 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).	Information on maize volunteers is not required in the comparative analysis of agronomic and phenotypic characteristics. Field observations indicate that maize grains may survive and overwinter in some EU regions, resulting in volunteers in subsequent crops (e.g. Gruber et al., 2008; Palaudelmàs et al., 2009; Pascher, 2016). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Thus, the establishment and survival of feral and volunteer maize in the EU is currently limited and transient.
Germany	BfN	II.4 Post-market monitoring on the genetically modified food or feed	The data provided to show the human and animal safety of MON87427xMON89034xMIR162¬xNK603 maize on the basis of its substantial equivalence to conventional maize (except for the introduced trait) are not sufficient. Therefore, a post-market monitoring for food and feed is required.	The GMO Panel concluded that the four-event stack maize, as described in this application, is nutritionally equivalent to and as safe as the non-GM comparator and the non-GM reference varieties tested. Four of the subcombinations have been previously assessed and no safety concerns were identified. The six subcombinations not previously assessed and included

			The applicant is further requested to explain how the PMM of the GMO in mixed GMO commodities imported, processed or used for food/feed is realised. This is requested because the monitoring of a GMO must be carried out on a case-by-case basis (Directive 2001/18/EC) with regard to species characteristics, modified traits, the intended use and the degree of exposition. Specific GM product quantities should be provided to estimate the degree of exposition. In case of mixed commodities, according to the precautionary principle, each imported and processed commodity must be assumed to contain any in EU approved GM maize and consequently all parameters identified for the different GM maize products should then be monitored.	in the scope of application EFSA-GMO-NL-2016-131 are expected to be as safe as and nutritionally equivalent to the single maize events, the previously assessed maize subcombinations and the four-event stack maize. Therefore, the GMO Panel considers that post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in application EFSA-GMO-NL-2016-131, is not necessary (see Section 3.4.3.7, 3.5.3 and 3.6.1 of the Scientific Opinion).
Germany	BfN	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	The occurrence of teosinte, a wild relative of maize, has been repeatedly reported in EU fields. As spillage of maize seed during transport is possible, the introgression of the Bt trait into teosinte has to be considered. Insect-resistance, however, may increase fitness in teosinte and thus the likelihood of the plant to become invasive or a pest problem (see II.1). Data both on the occurrence of teosinte and fitness consequences in teosinte after introgression of the Bt and HR trait are needed to finalize the risk assessment.	The potential of spilled maize grains to establish, grow and produce pollen is extremely low and transient. Therefore, 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, even if exposed to glyphosate containing herbicides or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins. The intended traits of the four-event stack maize will not allow to overcome other biological and abiotic factors limiting plant's persistence and invasiveness. It is unlikely that the intended traits of event the four event stack maize will provide a selective advantage to maize plants, except when they are exposed to glyphosate containing herbicides or infested by insect pests that are susceptible to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins.
Germany	BfN	II.5.3.4 Interactions of the GM plant with non-target organisms (NTOs)	a) Exposure analysis The scope of the application includes processing and the use for food and feed purposes. The main exposure route therefore will result from waste produced during processing and the use of the GMO as food and feed. However, information on the environmental exposure, which is a prerequisite for the assessment of effects on NTO, is incomplete. For Bt proteins an exposure route via manure from cattle fed with Bt maize has been demonstrated (Gruber et al. 2011; Gürtler et al. 2010). Paul et al. (2010) observed that	Considering the scope of application EFSA-GMO-NL-2016-131, which excludes cultivation, the environmental risk assessment of maize MON 87427 × MON 89034 × MIR162 × NK603 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed genetically modified (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 89034 × MIR162 × NK603 grains during transportation and/or processing (EFSA GMO Panel, 2010).

			44 % of the immunoreactive Cry1Ab from MON810 present in feed was transferred to the faeces (Paul et al. 2010) while 34 % of the Cry1Ab protein levels in feed could be detected in liquid manure (Gruber et al. 2011). As Gruber et al. (2011) demonstrated Cry1Ab is relatively stable in liquid manure (decrease of 49 % in 24 weeks). The bioactivity of Cry proteins in wastewater or manure is unknown as no bioassays have been carried out so far. Based on the above finding it is likely that Bt proteins present in MON87427xMON89034-xMIR162xNK603 maize (Cry1A.105; Cry2Ab2; Vip3Aa) will also be transferred from processing or feed directly or indirectly into the environment. Thus, the applicant should provide a detailed analysis on the fate of the Bt proteins in the environment and a quantitative estimate of subsequent exposure of non-target organisms. b) Effects on non-target organisms Based on the exposure analysis the applicant should provide data on the ecotoxicity of MON87427xMON89034xMIR162xNK603 maize to assess possible effects on non-target organisms and subsequent effects on biogeochemical processes. Little information on combinatorial effects between the different Bt proteins (or Bt proteins and other components such as HR) exist. As the outcome cannot be predicted a priori (De Schrijver et al. 2014; Hilbeck & Otto 2015) tests are necessary to address this issue. The scope of EFSA-GMO-NL-2016-131 does not include cultivation. Nevertheless, import and processing of the GMO may lead to environmental exposure via waste or faeces resulting from the use of the GMO as food or feed. Consequently, soil and water organisms are the most likely groups to be exposed to the novel proteins. Exposure routes, functional groups and test species should be selected according to an ecological test strategy (Hilbeck et al. 2008, 2014). Having collected data on the ecotoxicity the risk assessment should be updated including possible effects on soil and water organisms. A representative set of organisms with a high probability of exposure should be tested as Bt toxins are less specific than previously assumed and sensitivity of non-target organisms is difficult to predict (van Frankenhuyzen 2009, 2013; Hilbeck and Otto 2015). We recommend including water organisms in ecotoxicity testing. Several recent publications point at the presence of	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 89034 × MIR162 × NK603 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of maize MON 87427 × MON 89034 × MIR162 × NK603 with nontarget organisms are not considered by the GMO Panel to raise any environmental safety concern. The GMO Panel concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins is likely to be very low and of no relevance.

			Cry proteins and/or genes in aquatic systems and raise concerns about the safety of plant expressed Cry proteins to aquatic organisms (Bøhn et al. 2008, 2010, 2016; Douville et al. 2005, 2008; Prihoda & Coats, 2008; Rosi-Marshall et al. 2007). Bøhn,T., Primicerio,R., Hessen,D.O. & Traavik,T. (2008) Reduced Fitness of Daphnia magna Fed a Bt-Transgenic Maize Variety. Archives of Environmental Contamination & Toxicology, currently online (DOI 10.1007/s00244-008-9150-5). Bøhn,T., Traavik,T., Primicerio,R. (2010) Demographic responses of Daphnia magna fed transgenic Bt-maize. Ecotoxicology, 19, 419-430. 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, 130-140. De Schrijver, A., De Clercq, P., Booij, K., de Maagd, R. A., and van Frankenhuyzen, K. (2014) Can interactions between Bt proteins be predicted and how should effects on non-target organisms of GM crops with multiple Bt Proteins be assessed? Reserach report COGEM: CGM 2014-05, pp.1-94. Douville,M., Gagné,F., Masson,L., McKay,J. & Blaise,C. (2005) Tracking the source of Bacillus thuringiensis Cry1Ab endotoxin in the environment. Biochemical Systematics and Ecology , 33, 219-232. Douville,M., Gagné,F. & Blaise,C. (2008) Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (Elliptio complanata) near corn fields: Evidence of exposure by bacterial ingestion. Ecotoxicology and Environmental Safety (online), 1-9. 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.	

			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. Hilbeck,A., Weiss,G., Oehen,B., Römbke,J., Jänsch,S., Teichmann,H., Lang,A., Otto,M., Tappeser,B. (2014) Ranking matrices as operational tools for the environmental risk assessment of genetically modified crops on non-target organisms. Ecological Indicators, 36, 367-381. Hilbeck A., Jänsch, S., Meier M., Römbke J. (2008) Analysis and validation of present ecotoxicological test methods and strategies for the risk assessment of genetically modified plants. Federal Agency for Nature Conservation, Bonn - Bad Godesberg: 287 pp. (BfNSkript 236) http://www.bfn.de/fileadmin/MDB/documents/service/skript236.pdf Paul,V., Guertler,P., Wiedemann,S., 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. Prihoda,K.R. & Coats,J.R. (2008) Aquatic fate and effects of Bacillus thuringiensis Cry3Bb1 protein: toward risk assessment. Environmental Toxicology and Chemistry, 27, 793-798. Rosi-Marshall,E.J., Tank,L.J., Royer,T.V., Whiles,M.R., Evans-White,M., Chambers,C., Griffiths,N.A., Pokelsek,J., Stephen,M.L. (2007) Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings of the National Academy of Science USA, 104, 16204-16208. van Frankenhuyzen, K. (2009) Insecticidal activity of Bacillus thuringiensis crystal proteins. Journal of Invertebrate Pathology, 101, 1-16. van Frankenhuyzen, K. (2013) Cross-order and cross-phylum activity of Bacillus thuringiensis pesticidal proteins. Journal of Invertebrate Pathology, 114, 76-85.	
Germany	BfN	II.6 Post-Market Environmental Monitoring Plan (PMEM)	The scope of this application is for import, processing, and all uses for food and feed. The applicant provides an environmental monitoring plan, which remains very general. The 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	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.

			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 MON87427xMON89034xMIR162xNK603 maize will be transported, stored, packaged, processed or used for food/feed. In case of substantial losses and spread of MON87427xMON89034xMIR162xNK603 maize all receiving environments need to be monitored. The time period of monitoring needs to be sufficient to detect delayed or long-term adverse effects. Therefore it may be necessary to extend the monitoring regarding certain parameters beyond the period of consent. Since traders may commingle MON87427xMON89034xMIR162xNK603 maize with other commercial GM maize imported, processed or used for food/feed, the applicant is requested to explain how the monitoring will be designed to distinguish between potential adverse effects caused by MON87427xMON89034xMIR162xNK603 maize and those caused by other GM maize. There are gradual differences in the predictability among effects and therefore gradual transitions between case-specific monitoring and general surveillance. It is therefore necessary to include the option of investigating similar	

			parameters in case-specific monitoring, in general surveillance, or in both simultaneously. Consequently, some monitoring requirements are listed under both categories. The Federal Agency for Nature Conservation is of the opinion that a detailed monitoring plan has to be provided before consent may be given. 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. DOI: 10.1111/j.1523-1739.2009.01385.x	
Germany	BfN	II.6.1 Interplay between Environmental Risk Assessment, Risk Management and PMEM	The information necessary to conclude on the ERA is partly missing. Thus, the safety of MON87427xMON89034xMIR162xNK603 maize cannot be fully assessed. Depending on those results the conclusions concerning case-specific monitoring may need to be revised.	The GMO Panel considered that the information submitted by the applicant on application EFSA-GMO-NL-2016-131 was sufficient to conclude on the environmental risk assessment (ERA) of maize MON 87427 × MON 89034 × MIR162 × NK603. As the ERA did not identify potential adverse environmental effects from the four-event stack maize, the GMO Panel did not require case-specific monitoring.
Germany	BfN	II.6.2 Case Specific Monitoring (strategy, method and analysis)	We do 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 MON87427xMON89034xMIR162xNK603 maize enters the environment. Therefore the applicant is requested to provide an appropriate case-specific monitoring plan comprising at least the following elements: i.) spillage or loss of MON87427xMON89034xMIR162xNK603 maize during transport, storage, packaging, processing and use (feed and food), ii.) potential spread and persistence of MON87427xMON89034xMIR162xNK603 maize within all environments, where substantial amounts of viable MON87427xMON89034-xMIR162xNK603 maize are spilled, iii.) occurrence of teosinte in regions affected by transport, storage, packaging, processing and use (feed and food) and subsequently potential outcrossing of the transgenes, iv.) exposure of the different active toxins to the environment e.g. via sewage water, waste material, manure or by-products which may occur during processing or use of non-viable material of the GMO as food/feed, v.) environmental effects such as spread, persistence and accumulation of the active toxins in other organisms and environmental media.	Considering the introduced traits and the outcome of the comparative analysis, the routes of exposure and limited exposure levels, the GMO Panel concluded that the four-event stack maize would not raise safety concerns in case of accidental release of viable GM maize grains into the environment, irrespective of possible interactions between the individual events within maize MON 87427 × MON 89034 × MIR162 × NK603. There are no indications of an increased likelihood of spread and establishment of feral maize MON 87427 × MON 89034 × MIR162 × NK603 plants, unless these plants are exposed to the intended herbicides. Moreover, in light of the scope of the application, data available for one of the sub-combinations, the GMO Panel is of the opinion that any sub-combinations of the individual events, including those not previously assessed by EFSA, would raise no environmental safety concerns. As the environmental risk assessment did not identify potential adverse environmental effects from the four-event stack maize and the already assessed sub-combinations, the GMO Panel did not require case-specific monitoring.

			For parameters i.) to iii.), the use of the following methods is recommended (http://www.vdi.eu/engineering/vdi-standards/): o VDI-Guideline 4330 Part 10 "Floristic mapping of genetically modified plants their crossing partners and their hybrid offspring", o VDI-Guideline 4330 Part 5 "Guideline for the collection and preparation of plant samples for molecular biological analysis". If spread, persistence or accumulation of MON87427xMON89034xMIR162xNK603 maize or MON87427xMON89034xMIR162xNK603 maize products 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). MON87427xMON89034xMIR162xNK603 maize expresses three different proteins which are toxic to organisms. Furthermore MON87427xMON89034xMIR162xNK603 maize may enter the environment together with other approved GM maize lines containing different toxic proteins. Therefore, a special focus should be on potential effects on the environment based on the combination of several toxins. The control of adventitious maize plants and clean up measures are proposed to control spillage of viable plant material during transport, storage, packaging or processing. The Federal Agency for Nature Conservation is of the opinion, that these risk management measures should be confirmed as mandatory. Furthermore, the efficacy of the implemented risk management measures should be monitored during case specific monitoring (EFSA 2011). EFSA Scientific opinion. Guidance on the Post-Market Environmental monitoring (PMEM) of genetically modified plants. EFSA Journal 2011, 9(8): 2316, 40 pp.	
Germany	BfN	II.6.3 General Surveillance (strategy, method)	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	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.

			MON87427xMON89034xMIR162xNK603 maize 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 MON87427xMON89034xMIR162xNK603 maize. 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. We do not approve this, because processed material may also be a cause of adverse effects. Therefore, the applicant is requested to involve also operators further down the food chain in the process of monitoring. The general surveillance plan has to focus on possible pathways how MON87427xMON89034xMIR162xNK603 maize 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 MON87427xMON89034xMIR162xNK603 maize in environmental media. Besides 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. MON87427xMON89034xMIR162xNK603 maize may enter the environment together with other approved GM maize lines. Therefore, a special focus should be on possible combined effects.	

Germany	BfN	II.6.4 Reporting the results of PMEM	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 MON87427xMON89034xMIR162xNK603 maize imported into the EU, i.) the ports and silos where shipments of MON87427xMON89034xMIR162xNK603 maize were unloaded, ii.) the processing plants and users where viable MON87427xMON89034xMIR162xNK603 maize was transferred to, iii.) the amount of MON87427xMON89034xMIR162xNK603 maize used on farms for feed, and iv.) transport routes of MON87427xMON89034xMIR162xNK603 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	BVL (German CA)	II.1 Hazard identification and characterisation	The scope of application EFSA-GMO-NL-2016-131 covers import and processing of maize MON 87427 x MON 89034 x MIR162 x NK603 including all feed and food products containing, consisting of, or produced from the genetically modified maize MON 87427 x MON 89034 x MIR162 x NK603. Cultivation is not covered by this application. 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 MON 87427 x MON 89034 x MIR162 x NK603 is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended use. Nevertheless, completion and/or clarification on some points of the dossier are recommended.	The GMO Panel thanks Germany for this summary. Please see below for replies to specific comments.
Germany	BVL (German CA)	II.1.2.2 Information relating to the genetically modified plant	Overall, the presented data do not provide indications of unintended effects or interactions between the events in maize MON 87427 x MON 89034 x MIR162 x NK603 which may raise safety concerns. Nevertheless, we would like to comment on some minor points which should be addressed by the applicant (see comments below). II.1.2.2.2 Information on the sequences actually inserted/deleted or altered: With regard to the study TK0253806 A1 the applicant should be asked to confirm that the used test material,	TK0253806 A1 are two studies on the resequencing of the event MIR162 submitted in the frame of the stack application MON 87427 x MON 89034 x MIR162 x NK603. In TK0253806 A1 it is indicated that the test material (genomic DNA) is

			named ID 4xS_0904, is proven to be MON 87427 x MON 89034 x MIR162 x NK603. The study RAR-2015-0139) twice refers to a database named EST_2013 (see table of contents and headline to chapter 5.3.1) which seems out-dated according to its nomenclature. However, it could also be a typo, the more so as (CBI: RAR-2015-0139) refer to the database EST_2015 elsewhere in the text. This discrepancy should be clarified by the applicant. In order to get more detailed information on the used databases, (CBI: RAR-2015-0139) refer to themselves (circular reference in chapter 2 of RAR-2015-0139). In this regard, we would like to point to the submitted report (FROM CBI: MSL0026803) providing detailed information on the mentioned databases. According to the information given in the report MSL0026803) the timeliness of the databases should be specified with December 2014 (access to the GenBank data according to MSL0026803) instead of January 2015 ("database release date" in Appendix 3). The sequence analyses of event MON 87427, event MON 89034 and event NK603 were performed using identical databases and algorithms, whereas sequence analysis of event MIR162 was partly carried out using different databases and algorithms. Ideally, bioinformatics analyses of all events should be uniform. II.1.2.2.4 Genetic stability of the insert and phenotypic stability of the genetically modified plant: The DNA sequences of the inserts as well as the flanking genomic regions were analysed by PCR and subsequent sequencing of the PCR products. The presence of the four inserts in line MON 87427 x MON 89034 x MIR162 x NK603 was determined by comparative PCR analyses with inserts present in the underlying single events. These studies confirmed a base exact match between the respective sequences in the stacked event MON 87427 x MON 89034 x MIR162 x NK603 and in the four parental lines. However, it must be noted that the used PCR methodology is not suitable to detect possible - albeit unlikely - duplications of an insert or parts of it. Even though the probability of such an event is considered low, the applicant should be responsive to this question.	extracted from ground pooled maize seeds of a sample identified with the ID 4xS_0904. In Appendix B (page 39) of the same study, the description of the sequence alignment indicates that the test material 4xS_0904 is actually the combined trait product, confirming that 4xS_0904 is indeed the stack material MON 87427 x MON 89034 x MIR162 x NK603. The GMO Panel took the comment into account. The applicant provided updated bioinformatics analysis for all events that was conducted according to EFSA guidelines. The GMO Panel assessed the new information which confirmed the previous conclusions, and do not raise safety concerns. Data on the genetic stability over several generations have been provided in the single applications and have been previously assessed by the GMO Panel. The applicant provided two additional studies, MSL0026686 and MSL0026201, including Southern blot analysis for all the events in the stack. These studies provided additional evidences that the events in the stack material are in single copy and that the insertion site in the plant genome has been retained between the stack and the respective single. Furthermore, the sequence analysis of the MON 87427 x MON 89034 x MIR162 x NK603 showed that the inserts have retained their integrity. The sequence analysis performed on all four events of the maize stack MON 87427 x MON 89034 x MIR162 x NK603 has been performed according to the

			II.1.2.2.5 Potential risk associated with horizontal gene transfer: In order to get more detailed information on the databases used to provide information on the similarities of DNA sequences inserted into the genome of maize MON 87427 x MON 89034 x MIR162 x NK603 with microbial DNA sequences (FROM CBI: MSL0026583) refer to another study. However, this study is not part of the application documents. Therefore, the applicant should clarify if he refers to the information given in the report of (FROM CBI: MSL0026803) where the timeliness of the databases BCT_2015, PLS_2015 and VIR_2015 is specified with December 2014.	applicable EFSA guidance document (EFSA GMO Panel, 2011a) and is considered sufficient by the GMO Panel to conclude on the integrity of the inserts. An updated bioinformatics analysis was received from the applicant in March 2019. The new bioinformatics analysis contains the assessment of potential horizontal gene transfer for all the events of the stack (MON 87427, MON 89034, MIR162 and NK603) and was generated using up-to-date databases and algorithms. The analysis was performed in line with the requirements of the GMO Panel guidelines on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a) and the explanatory note on horizontal gene transfer (HGT; EFSA, 2017b). The outcome of the new HGT analysis did not identified new hazards and hence confirmed the previous conclusions.
Germany	BVL (German CA)	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	In section 3.2 of Study # SCR-2014-0463 (FROM CBI: MSL0027656) the applicant states that the identities of the test and control starting seed were verified by event-specific PCR analyses and that all test and control starting seed materials had acceptable purity level and are deemed appropriate by the lead scientist. In this regard, the applicant should be requested to provide these purity levels.	As described in reports SCR-2014-0463 and REG-2013-0630, the identity of maize MON 87427 x MON 89034 x MIR162 x NK603 and of its comparator was tested via event specific PCR and the materials were considered of acceptable purity levels. The quality of the non-GM reference varieties is guaranteed by the corresponding producers. Following a request of the GMO Panel, the applicant provided further information to characterise the quality of the starting materials (additional information received on 29/9/2016). In conclusion, the GMO Panel considered that the information available was adequate for the risk assessment of the four-event stack maize.
Germany	BVL (German CA)	II.1.3.5 Comparative analysis of agronomic and phenotypic characteristics	The data provided in tables 9 and 10 of Part II of the application (Scientific Information) show inconsistencies to the given reference of the actual study (FROM CBI: MSL0027656, tables 9 and 10). These different values/numbers do not alter the risk assessment, though.	The GMO Panel thanks Germany for this comment.
Germany	BVL (German CA)	II.5.3.1 Persistence and invasiveness including plant-to-plant gene flow	The import documents should indicate that maize MON 87427 x MON 89034 x MIR162 x NK603 has not been approved for cultivation by the EC. In addition to the intended GM labelling, a clear labelling of maize MON 87427 x MON 89034 x MIR162 x NK603 indicating the tolerance to glyphosate is recommended. Furthermore, appropriate measures have to be taken during transport, storage, and processing to avoid unintended release of germinable	The GMO Panel is aware that, owing to the physical characteristics of maize grains and methods of transportation, accidental spillage cannot be excluded. Hence, it is important that appropriate management systems are in place to restrict grains of maize MON 87427 x MON 89034 x MIR162 x NK603 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

			maize kernels into the environment. In this context, the applicant should inform all parties involved in the handling and processing of maize MON 87427 x MON 89034 x MIR162 x NK603 about avoidance and control of spillage.	
Germany	BVL (German CA)	II.6 Post-Market Environmental Monitoring Plan (PMEM)	The monitoring plan is acceptable, but needs further elaboration for implementation. Therefore, the applicant is recommended to revise the monitoring plan during the initial implementation phase (after consent is given) and present this revised monitoring plan together with a first report one year after consent is given to be reassessed.	Monitoring 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	BVL (German CA)	II.6.2 Case Specific Monitoring (strategy, method and analysis)	According to the risk assessment, no adverse effects on the environment or human health were identified or were expected. Therefore, there is no necessity for a case-specific monitoring.	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	BVL (German CA)	II.6.3 General Surveillance (strategy, method)	The monitoring plan does not relate the monitoring activities to relevant protection goals. Even more, it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter, because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate food and feed surveillance in coordination with the competent authorities. Information about the use of the product in food and feed could deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Therefore, the applicant should specify monitoring activities in the field of human and animal health. He should describe in detail how animal and human health surveillance is integrated in the monitoring plan. 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	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.

			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 ongoing research, should be taken into account. However, the applicant should describe in detail how he would consider this information within General Surveillance.	
Germany	BVL (German CA)	II.6.4 Reporting the results of PMEM	A report on General Surveillance activities on an annual basis is sufficient. Reporting should refer to the format introduced by the Commission Decision 2009/770/EC. The applicant is requested to state how the monitoring results will be published.	Monitoring 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	BVL (German CA)	II.7 Additional information related to the safety of the genetically modified food or feed	The applicant gives a comprehensible explanation why it is not practically feasible to perform a systematic review in the present case. Nonetheless, in Table 21, the applicant provides a compilation of studies on potential effects of MON 87427 x MON 89034 x MIR162x NK603 on human and animal health that were performed by the applicant within the period of ten years prior to the date of submission of this application. Due to an EFSA GMO Panel request the applicant provided the full reports of the studies listed in Table 21. For the sake of clarity, the applicant might be asked to present the studies and their results in the form of a summary report.	The GMO Panel took note of the comment.
Hungary	Ministry of Agriculture	II.1.2.1 Information relating to the genetic modification	1.2.1.3. (b) It is stated, that the transgenic proteins expressed in MON87427×MON89034×MIR162 ×NK603 maize have a history of safe consumption. It is not so. The transgenes (the 2 versions of the CP4 EPSPS and the CP4 EPSPS L214P7 proteins, the Cry1A.105 and Cry2Ab, the Vip3Aa20, and the PMI proteins) have been modified and/or optimised to be expressed in plants and are different from the native proteins. They also have different regulatory elements attached to them. Neither the native nor the transgenic proteins were used as food or feed before. Although the transgenic proteins have been a part of the food/feed supply for about 10 years, it cannot be considered as	The GMO Panel took note of the comment. The safety of the proteins newly expressed in GM maize MON 87427 x MON 89034 x MIR162 x NK603 has been previously assessed by the GMO Panel in the context of the single events, and no safety concerns were identified for humans and animals (EFSA 2004, 2007, 2008, 2009; EFSA GMO Panel, 2012, 2015b). The assessment was based on a weight of evidence approach considering the structure and function of the new proteins, the results of bioinformatic analysis showing no identity to toxins, the results on in vitro digestibility studies. The GMO Panel is not aware of any new information that would change this conclusion, in particular as

			"history". In addition, there is no way to know if they have/had any harmful effect(s), since no one know who has consumed what transgenic proteins, when and in what amounts. Although maize has a history of safe consumption, but does not mean that any stacked GM maize variety has a history of safe use. The combination of the individual transgenes has never occurred together in Nature, or in the food/feed before, therefore the combination has no history of safe use, or any other type of safety. There is no sufficient proof to show that these proteins are safe and pose no concerns for humans, animals or the environment. This is especially true for the Cry proteins, which have proven risks when consumed in combination (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140). It was concluded that Cry-toxins in combination indicate alternative modes-of-action. The authors suggested that 'stacked events' may have stronger effects on non-target organisms, and that further studies are need to be done on the combinatorial effects of multiple Cry-toxins and herbicides that co-occur in the environment....). (e) The history of the donor organisms is questionable, since none of the donor organisms were used as food or feed. In addition, the transgenes are modified versions of the natural genes, which have never been tested for safety from the GM plant itself. The CP4 EPSPS proteins confer tolerance to the application of glyphosate herbicide. The question arises if the level of glyphosate and its metabolites in the maize kernels and other parts of the plant (such as silage) are in the range permitted, or are higher. Therefore, Hungarian experts respectfully suggest measuring the levels of these chemicals in every shipment of market MON 87427 × MON 89034 × MIR162 × NK603, and its relevant sub-stacks, when received by the EU.	regards the mode of action of the newly expressed proteins and its relevance for humans and animals (see Section 3.4.3.3 of the Scientific opinion for further details). The assessment of herbicide residues and metabolites is not in the remit of the GMO Panel.
Hungary	Ministry of Agriculture	II.1.2.2 Information relating to the genetically modified plant	1.2.2.2 (a) It is stated in the Dossier that "there is low likelihood of molecular interactions between the inserts from MON 87427, MON 89034, MIR162 and NK603 and therefore, low likelihood of any changes in the molecular characteristics of the inherited inserts in MON 87427 × MON 89034 × MIR162 × NK603", but no experimental proof (no full length DNA	The sequence of each insert has been newly determined in the four event stack maize MON 87427 × MON 89034 × MIR162 × NK603. These data showed that there no changes in the sequence of the inserts compared to the singles which have been previously assessed. Furthermore, the compositional data do not

			analysis, only partial) is provided to support this statement, and furthermore, nothing to prove that there is no interaction between the transgenic proteins. Although the inserts are present at different genetically loci, and the likelihood of molecular interactions between the different inserts is low, there might be interactions between the transgenic gene products, the individual transgenic proteins. For example, it is feasible to conclude, that the expression of multiple cry proteins has an additive effect not only on plant protection but on target- and none target organisms, as it is described in the scientific literature. It is also likely that those proteins have multiple receptors and different effects/modes of actions in vivo on humans and animals consuming those cry proteins simultaneously, as it has been demonstrated by Bohn et al. (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140). It was concluded that Cry-toxins in combination indicate alternative modes-of-action. The authors suggested that 'stacked events' may have stronger effects on non-target organisms, and that further studies are need to be done on the combinatorial effects of multiple Cry-toxins and herbicides that co-occur in the environment).	indicate any significant changes that could be a result of a molecular interaction that would raise a safety concern. 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 89034 × MIR162 × NK603 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of maize MON 87427 × MON 89034 × MIR162 × NK603 with nontarget organisms are not considered by the GMO Panel to raise any environmental safety concern. The GMO Panel concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry2Ab2 and/or Vip3Aa20 proteins is likely to be very low and of no relevance.
			1.2.2.3 (a) Which proteins were the individual antibodies used in ELISA raised against, the protein present in the GM plant, or against the bacterial recombinant version of the proteins? In Table 2 the CP4 EPSPS protein levels in maize tissues collected from MON 87427 × MON 89034 × MIR162 × NK603, MON 87427 and NK603 are very different, see forage R5:74-120 and 31-69 or 15-33; in grain R6: 8.4-16 and 1.8-8.0 or 4.0-7.6.	The biochemical and functional equivalence between plant and microbe-derived newly expressed proteins was sufficiently demonstrated in the applications of the singles which have already been assessed. The GMO Panel therefore considers that in this respect, the expression system used to extract the proteins from would not influence the ELISA method. The four event maize stack MON 87427 × MON 89034 × MIR162 × NK603 contains two single events expressing the CP4 EPSPS protein and it is therefore not unexpected that the stacked event may express higher amounts of the CP4 EPSPS protein. In addition, the observed differences are not large enough to indicate that there would be an interaction affecting protein levels.
			1.2.2.5 Horizontal gene transfer of plant DNA sequences to human or animal cells had been recorded in the scientific literature (Rizzi et al., 2012).	The GMO Panel took note of the comments raised by Hungary and wishes to clarify that besides exposure it also considered the consequences of an unlikely but theoretically possible HGT. The

			Although natural barriers exist to inhibit the incorporation of foreign DNA to the genome, some nucleic acids can be taken up by cells. It appears that stomach acid, pancreatic nucleases, intestinal epithelium, vascular endothelium and blood/systemic nucleases, and cellular barriers that include the plasma membrane, endosomal sequestration and lysosomal degradation still allow some nucleic acids to enter systemic circulation and are able to pass through the placenta and even the blood brain barrier (Dörfler et al, 2001). A study conducted on human subjects fed on genetically modified soybean has shown that a proportion of the full length of the plant transgene does survive passage through the human gastro- intestinal tracts, and evidence suggests that gene transfer actually occurred between GM soybean and intestinal micro-flora during the experiments (Netherwood et al., 2004). Indeed, the study has shown that the full length of the transgene, although in small quantities, survived digestion and could be detected from samples of microbes taken from the ileostomy bag (of microbes resident in the gut). Therefore, the possibility of horizontal gene transfer from the GM plants to gut microbes is quite likely in human or in animals. Mammals have been shown to take up dietary DNA from the gastrointestinal tract (Rizzi et al., 2012). A study cited in the Dossier about human subjects being fed on genetically modified maize does not exist. That experiment has been performed with RoundUp-Resistent GM soy, and it has confirmed that the full transgene survived passage through the human gastro-intestinal tracts and was found to be transferred to the intestinal micro-flora, although in small quantities (Netherwood et al., 2004).	updated bioinformatics analyses of events MON 87427, MON 89034, MIR162 and NK603 do not reveal any new DNA sequence that could provide sufficient length and identity which could facilitate HGT by double homologous recombination, confirming the previous conclusions (EFSA GMO Panel, 2017a,b, 2019a,b,c). In summary, there is no indication for an increased likelihood of horizontal transfer of DNA from event MON 87427, MON 89034, MIR162 and NK603 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT. The updated bioinformatic analysis has been conducted by the applicant in line with the latest EFSA requirements. In addition to the consideration for the likelihood of recombination, the risk assessment also includes the identification of potential hazards caused by the transfer of the genetic elements of bacterial origin from the GM plants to environmental bacteria. In the case of stack maize MON 87427 × MON 89034 × MIR162 × NK603 it is unlikely that an unlikely but theoretically possible HGT will confer a selective advantage to recipients.
Hungary	Ministry of Agriculture	II.1.2.4 Conclusions of the molecular characterisation	1.2.4 Although there is no evidence of interaction between the different transgenes inserted to MON 87427 × MON 89034 × MIR162 × NK603 maize, there is evidence of interaction between the different transgene products, especially between the Cry proteins (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140). The potential risk associated with horizontal gene transfer to occur from MON 87427 × MON 89034 × MIR162 ×	For the interaction between Cry proteins, please see the response below (comment on II.1.4.1). For HGT, please see the response to the previous comment.

			NK603 to humans, animals or micro-organisms is extremely likely, which is ignored by the company.	
Hungary	Ministry of Agriculture	II.1.3.1 Choice of the conventional counterpart and additional comparators	1.3.1 When assessing the safety of a new GM line only the parent/conventional comparator and the GM plant should be compared. The use of commercial maize varieties are unnecessary and even misleading. To show biological variation, the use of a single commercial maize variety is sufficient, but not obligatory. Both the GM and the non-GM lines provide a range of "natural variation".	The field trial design and the statistical analysis were in line with the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a). As explained e.g. in EFSA GMO Panel (2010b), multiple sources of natural variation should be considered: 'natural variation is the variability occurring naturally because of differences in the genotypes of plants, effects of environmental factors and the interaction between them'. Variability occurring 'because of differences in the genotypes of plants' is accounted for by the commercial reference varieties grown in the field trials.
Hungary	Ministry of Agriculture	II.1.3.2 Experimental design and statistical analysis of data from field trials for comparative analysis	1.3.2.1 The use of a total of 17 unique, conventional commercial reference maize varieties to provide phenotypic and environmental interaction characteristics values representative of commercial maize has no relevance to the safety of MON 87427 × MON 89034 × MIR162 × NK603 maize. Indeed it is used to widen the natural range of the compounds.	The field trial design and the statistical analysis were in line with the recommendations of the GMO Panel (EFSA GMO Panel, 2010b, 2011a).
Hungary	Ministry of Agriculture	II.1.3.4 Comparative analysis of composition	1.3.4.2 Statistically significant differences were found in NT grain between the GM- and its conventional comparator for palmitoleic acid, arginine, cystine/cysteine. Statistically significant differences were found in T grain between the GM- and its conventional comparator for palmitoleic acid, arginine, cystine/cysteine, tryptophan, phosphorus. Therefore, the two maize varieties are different.	The GMO Panel assessed all significant differences between the four-event stack maize and the non-GM comparator, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. The GMO Panel concluded that none of the differences needed further assessment for food/feed safety (Scientific Opinion, Section 3.4.2).
Hungary	Ministry of Agriculture	II.1.3.6 Effects of processing	1.3.6 Evidence suggests that processing does not degrade some of the transgenic proteins.	When estimating human and animal dietary exposure to the different newly expressed proteins (CP4 EPSPS (CP4 EPSPS and CP4 EPSPS L214P), Cry1A.105, Cry2Ab2, Vip3Aa20, and PMI proteins) it is assumed that processing does not affect proteins, and the same amount present in the raw primary commodity is present in the processed commodity.
Hungary	Ministry of Agriculture	II.1.4.1 Testing of newly expressed proteins	1.4.1 None of the transgenic proteins have history of safe use. The donor organisms have never been consumed as food/feed before. All transgenes are synthetic forms of the genes occurring in Nature. They are modified, synthetic versions of the gene from the donor organisms, in a different regulatory million, including promoters, artificial introns, stop signals and so on. According to Hungarian experts, the safety of the Cry,	The GMO Panel took note of the comment. The assessment of the proteins newly expressed in maize MON 87427 x MON 89034 x MIR162 x NK603 has been previously assessed by the GMO Panel in the context of the single events, and no safety concerns were identified for humans and animals. The GMO Panel is not aware of any new information that would change this conclusion. In particular,

			Vip3Aa20, PMI or CP4 EPSPS proteins has not been proven previously, and the safety of all these proteins in one crop needs proper toxicological evaluation, considering the possible interaction between these transgenic proteins, as well as between the proteins and the herbicide(s) residues and metabolites used on them. The expression of multiple cry proteins has an additive effect on plant protection. It is also likely that those proteins have multiple receptors and different effects/modes of actions in vivo on humans and animals consuming cry proteins simultaneously, as it has been demonstrated by the paper of Bohn et al (2016). It was concluded in their paper that Cry-toxins in combination indicate alternative modes-of-action. The authors suggested that 'stacked events' may have stronger effects on non-target organisms, and that further studies are need to be done on the combinatorial effects of multiple Cry-toxins and herbicides that co-occur in the environment. Based on these comments Hungarian experts suggest to perform animal feeding study(s) with rodents to assess reproductive, developmental and chronic toxicity, as well as food/feed safety of MON 87427 × MON 89034 × MIR162 × NK603 maize. 1.4.1.4 It cannot be stated, since it was not proven, that the transgenic proteins expressed in MON 87427 × MON 89034 × MIR162 × NK603 stack GM maize have a history of safe consumption as part of approved single GM events that are grown in the U.S. and other regions. 10 years or so cannot be called history, and these transgenic Cry1F, PAT, Cry1Ab, Vip3Aa20, PMI and CP4 EPSPS proteins have been a part of the food supply only for a few years. One cannot say that these transgenic proteins were consumed without incident and that they pose no significant risk of adverse toxic effects, since GM food in the US is not labelled, no one knows what amount of, and what type of the transgene(s) was consumed by whom and when. Based on these comments Hungarian experts suggest that there was an urgent need to perform animal feeding study(s) with rodents to assess reproductive, developmental and chronic toxicity, as well as food/feed safety of MON 87427 × MON 89034 × MIR162 × NK603 maize. The genes present in GM plants are different from the natural plant genes, since their sequence has been modified and/or are coupled with different regulatory elements. Our	the GMO Panel has assessed the papers quoted by Austria and found these not impacting the previous assessment on these proteins. As regards interactions, the GMO Panel is of the opinion that there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize considering the known biological function of the individual newly expressed proteins. Therefore, no additional studies on these proteins, individually or in combination, are considered necessary by the GMO Panel. Please see Section 3.4.3.3 of the Scientific Opinion for further details; see also the reply to the previous comment from Hungary. As regards animal studies on the four stack-maize, based on the outcome of the molecular characterisation assessment, comparative analysis and toxicological assessment, no indication of findings relevant to food/feed safety have been identified related to the stability and expression of the inserts or to interaction between the transformation events, and no modifications of toxicological concern have been identified in the composition of the four-event stack maize. Therefore, in line with EFSA GMO Panel (2011a) and Regulation (EU) 503/2013 animal studies on food/feed derived from the four-event stack maize are not necessary. The assessment of herbicides and their metabolites is not in the remit of the GMO panel.

			gut has never been exposed to such synthetic DNA sequencing before. Every cell is capable to take up sequences of RNA and DNA of differing length, so do microbes, although these sequences do not enter the germ cells. The CP4 EPSPS protein might not have synergistic or antagonistic effects with the other transgenes present in MON 87427 × MON 89034 × MIR162 × NK603 maize, but the herbicide the CP4 EPSPS protein provides tolerance for does have an effect(s), and so has its residue(s) and metabolites on the gut flora. 1.4.1.5 Although all of the introduced traits from the parental lines are inherited by the MON 87427 × MON 89034 × MIR162 × NK603 progeny, but the combined expression of the CP4 EPSPS, CrylA.105, Cry2Ab2, Vip3Aa20 and PMI proteins in the same plant but it had not been demonstrated that the CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins and their source organisms have a history of safe consumption, or that those organisms have been consumed as food/feed previously. In addition, the modes of action of the Cry1A.105, Cry2Ab2, and Vip3Aa20 proteins have not been investigated in mammals, although there are indications of synergistic effects to each other (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140). It is argued that based on weight of evidence there is no need to perform toxicological experiments. However, the arguments used are not supported by experimental evidence. It is stated that, 1) there is a history of safe use of the CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins and their source organism; but there is none; 2) the lack of structural or functional relationship of CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI to proteins that adversely affect human or animal health; which is not supported by experimental data; 3) the negligible human exposure to CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins from maize consumption; which ignores that small amount of these proteins in combination can have a significant biological effect;	

			4) there are no data to indicate full digestibility of CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins in vivo; or 5) that the CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins e deactivate upon heat treatment.	
Hungary	Ministry of Agriculture	II.1.4.4 Testing of the whole genetically modified food or feed	1.4.4.1 It is stated by EFSA that "an additional 90-day feeding study with whole food and feed in rodents with the genetically modified plant with the stacked transformation events shall be included where indications of potential adverse effects are identified during the assessment of: (i) the stability of the inserts; (ii) the expression of the inserts; and (iii) the potential synergistic or antagonistic effects resulting from the combination of the transformation events". There are indications of interaction between the cry proteins (Thomas Bøhn, Carina Macagnan Rover, and Philipp Robert Semenchuk (2016) Daphnia magna negatively affected by chronic exposure to purified Cry-toxins. Food and Chemical Toxicology 91: 130-140) and perhaps also with the Vip3Aa20. Therefore, a 90-day feeding study with whole food and feed in rodents with the genetically modified plant with the stacked transformation events should have been included in the Dossier.	The GMO Panel took note of the comment. See the reply to the previous comments from Hungary.
Hungary	Ministry of Agriculture	II.1.4.5 Conclusion of the toxicological assessment	1.4.5 The opinion of the Hungarian experts is that no sufficient proof has been provided to convince them that MON 87427 × MON 89034 × MIR162 × NK603 maize is safe to eat as food and/or feed.	The GMO Panel took note of the comment. Please see the replies above.
Hungary	Ministry of Agriculture	II.1.5.1 Assessment of allergenicity of the newly expressed protein	1.5.1 No proof is provided that the transgenic proteins in MON 87427 × MON 89034 × MIR162 × NK603 maize degrade fully in vivo. 1.5.1.3. No proof is provided that the transgenic proteins in MON 87427 × MON 89034 × MIR162 × NK603 maize degrade fully in vivo. 1.5.1.4 The opinion of the Hungarian experts is that no sufficient proof has been provided to convince them that MON 87427 × MON 89034 × MIR162 × NK603 maize is safe to eat as food and/or feed.	The allergenicity assessment of this GM maize has been performed following the relevant GMO Panel guidance documents and Codex Alimentarius guidelines. The GMO Panel has previously evaluated the safety of the Cry1A.105, Cry2Ab2, PMI, Vip3Aa20 and CP4 EPSPS (including its variant CP4 EPSPS L214P) proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed. No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. In the context of this application and considering the data from the molecular characterisation, the compositional analysis and the assessment of the newly expressed proteins, the GMO Panel identified no indications of a potentially increased allergenicity of food and feed derived from the four-event stack

				maize with respect to that derived from the non-GM comparator.
Hungary	Ministry of Agriculture	II.1.6 Nutritional assessment	6 The monitoring plans for MON 87427 × MON 89034 × MIR162 × NK603 maize maize is the same as for all other GM plants, including the same problems. No monitoring is carried out by independent observers and although the questioners are filled by operators, they are not available for inspection. Present methods used in Post Market Monitoring are not suitable to identify any risks. Even if any effects would be observed during monitoring, it would be impossible to tie those effects to any GM crops. Routine monitoring is conducted as a precaution and to detect unforeseen effects. The real question is, is there any effect which can be detected by general monitoring, when so many different GMO are in the feed and food supply? How can an effect, if found tied to any GM crop?	Comparison of the composition of maize MON 87427 × MON 89034 × MIR162 × NK603 with the non-GM comparator and non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that the nutritional impact of maize MON 87427 × MON 89034 × MIR162 × NK603-derived food and feed is the same as that expected from the non-GM comparator and non-GM reference varieties. The GMO Panel considers that post-market monitoring of food and feed from the four-event stack maize and its subcombinations, as described in this application, is not necessary.
Hungary	Ministry of Agriculture	II.1.6.3 Conclusion of the nutritional assessment	1.6.3 The opinion of the Hungarian experts is that no sufficient proof has been provided to convince them that MON 87427 × MON 89034 × MIR162 × NK603 maize is safe to eat as food and/or feed.	Comparison of the composition of maize MON 87427 × MON 89034 × MIR162 × NK603 with the non-GM comparator and non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that the nutritional impact of maize MON 87427 × MON 89034 × MIR162 × NK603-derived food and feed is the same as that expected from the non-GM comparator and non-GM reference varieties.
Hungary	Ministry of Agriculture	II.2 Exposure assessment — anticipated intake or extent of use	2.4 The exposure assessment ignores the fact that a great many people are intolerant or allergic to wheat gluten and forced to switch and eat maize. Their number increases year by year. Instead of using wheat those persons use maize flour for cooking and baking.	Human dietary exposure to the different newly expressed proteins [CP4 EPSPS (CP4 EPSPS and CP4 EPSPS L214P), Cry1A.105, Cry2Ab2, Vip3Aa20, and PMI proteins] was estimated considering a 100% replacement scenario that is considered conservative when assessing potential risks linked to the intake of these proteins. Additionally, potential losses of the newly expressed proteins during processing were not considered, which also implies an overestimation of the actual dietary exposure.
Hungary	Ministry of Agriculture	II.5 Environmental risk assessment	5 Exposure through faeces of animals fed to the GM maize can have an effect on the environment and non-target organisms, and this was not considered in the risk assessment. In addition glyphosate affects soil microbe composition, according to Bedano and Domínguez (José Camilo Bedano and Anahí Domínguez (2016) Large-Scale Agricultural Management and Soil Meso- and Macrofauna Conservation	Considering the scope of application EFSA-GMO-NL-2016-131, which excludes cultivation, the environmental risk assessment of maize MON 87427 × MON 89034 × MIR162 × NK603 mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed genetically modified (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 89034 ×

			in the Argentine Pampas. Sustainability 8: 653-678; doi:10.3390/su8070653).	MIR162 × NK603 grains during transportation and/or processing (EFSA GMO Panel, 2010a). Given that environmental exposure of non-target organisms to spilled GM grains or occasional feral GM maize plants arising from spilled four event stack maize grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, potential interactions of the four event stack maize with non-target organisms are not considered by the GMO Panel to raise any environmental safety concern. Interactions that may occur between the Cry or Vip proteins will not alter this conclusion.
Hungary	Ministry of Agriculture	II.7 Additional information related to the safety of the genetically modified food or feed	7 The outcome of systemic review strictly depends on the selection criteria, and if those are faulty, or certain method are excluded it changes the outcome of the review. If only articles related to risks are considered, and "a compilation of studies performed under direct control of the applicant ", it is not surprising, that no studies were found. Monsanto technical reports do not count as refereed scientific publication. Since independent researchers do not have access to research material, there are no independent safety studies to prove that MON 87427 × MON 89034 × MIR162 × NK603 maize is safe and poses no risk to human or animal health or the environment.	The GMO Panel assessed the systematic literature searches provided by the applicant, as well as additional information on the search strategy, as requested by EFSA, in accordance with the recommendations on literature searching outlined in EFSA (2010, 2017a). The overall quality of the performed literature searches is acceptable; however, the GMO Panel considered that future searches on maize MON 87427 × MON 89034 × MIR162 × NK603 should be improved. The GMO Panel provided specific recommendations (please refer to Section 3.1 of the Scientific Opinion).
Hungary	Ministry of Agriculture	Part I – General information	General comments: The Hungarian Authority has already asked for all relevant information to be presented in the applications, without referring to earlier applications. Hungary objected to the authorisation of the individual GM lines present in MON 87427 × MON 89034 × MIR162 × NK603 maize and all of their combinations, and strongly opposes the authorisation of MON 87427 × MON 89034 × MIR162 × NK603 maize as well, on strictly scientific basis.	The GMO Panel took note of the comment.
Italy	Ministero dell'Ambiente	II.6 Post-Market Environmental Monitoring Plan (PMEM)	As described by the EFSA guidance on PMEM (EFSA Panel on Genetically Modified Organisms, 2011), "GS plans should include questionnaires to those involved in the handling and processing of the GMP and its products and be designed to monitor whether unanticipated levels of loss, spillage and establishment are occurring and/or if there are any adverse environmental consequences". Nowhere in the PMEM proposed by the applicant were described questionnaires to the operators involved, nor how these questionnaires are	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.

			structured, which information collect and how this information will be analyzed: it is required to provide this information. "Approach": the applicant refers only to substantial unintended losses of GM maize during loading/unloading of the viable commodities as a route for environmental exposure. Other routes of exposure of the environment (e.g. waste materials from processing or use of GM maize, transportation) were not assessed specifically. The applicant should analyze all potential routes of exposure, including waste material and transportation. Moreover, the notifier states that "Exposure can be controlled by clean up measures and the application of current practices used for the control of any adventitious maize plants, such as manual or mechanical removal and the application of herbicides (with the exception of glyphosate or glufosinate herbicides)". No clear responsibilities are assigned in case of accidental exposure, so it remains unclear who actually will be responsible for those clean-up measures: we ask to detail more this aspect. Lastly, according to the applicant, the operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable MON87427×MON89034×MIR162×NK603 maize: it is required to provide such guidelines to evaluate their effectiveness.	
Italy	Ministero dell'Ambiente	II.6.3 General Surveillance (strategy, method)	II.6.3 General Surveillance - "Strategy":: the applicant is working together with other members of the plant biotechnology industry within the European Association of Bioindustries (EuropaBio) and trade associations representing the relevant operators in order to implement an harmonised monitoring methodology. Not all European Member States are represented within these associations: therefore, it would be appropriate to provide explanations on the monitoring methodology adopted in the MS not represented. "Methodology" - the applicant states that the information collected will be evaluated and analyzed in order to assess the relevance: the method is not specified and then it is required to provide it. In the EFSA guidance on PMEM (EFSA Panel on Genetically Modified Organisms, 2011) is established that "In addition, applicants should provide raw data in order to allow different analyses and interrogation of the data and to allow scientific exchange and co-operation between applicants, Member States, the	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.

			European Commission and EFSA": then, it would be appropriate that the applicant provides also the raw data, as well as the analyzes. Lastly, the notifier says that "Where information indicates the possibility of an unanticipated adverse effect, the authorisation holder will immediately investigate to determine and confirm whether a significant correlation between the effect and MON87427×MON89034×MIR162×NK603 can be established": we ask to specify the investigation method.	
Italy	Ministero dell'Ambiente	II.6.3 General Surveillance (strategy, method)	In the paragraph it is stated that "The operators will be provided with guidance to facilitate reporting of any unanticipated adverse effect from handling and use of viable MON 87427 × MON 89034 × MIR162 × NK603". In order to better evaluate the proposed general surveillance plan, it could be useful to know the content of the above mentioned guidance because it is right during the handling of goods that unintended release into the environment can occur.	Monitoring 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.
Italy	Ministero dell'Ambiente	II.6.4 Reporting the results of PMEM	Reporting the results of PMEM": it would be useful include in the annual monitoring report for the MON87427×MON89034×MIR162×NK603 maize information on foreseen amount of imported maize into the EU, ports, silos and processing facilities where the viable GM maize will be loaded/unloaded and transferred to, and transportation routes. In addition, it is advisable to specify in this paragraph if the annual report also contains the results of the screening of peer-reviewed publications conducted by the notifier (referred to in par. 6.4.5). Referenze/References: • EFSA Panel on Genetically Modified Organisms, 2010. Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879. • EFSA Panel on Genetically Modified Organisms, 2011. Guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA Journal 2011;9(8):2316. • EFSA Panel on Genetically Modified Organisms 2011. Scientific Opinion on Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 2011; 9(5): 2150.	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.
Netherlands	Dutch GMO Office	Part I – General information	- The Dutch CA has assessed the dossier with respect to the environmental, food and feed safety of event	The GMO Panel thanks the Netherlands for this assessment.

			MON87427 x MON89034 x MIR162 x NK603 maize and has no comments or requests for additional information in relation of the safety of the GM event.	
Norway	Norwegian Environment Agency	II.1.5.1 Assessment of allergenicity of the newly expressed protein	From the Application, it seem as none of these analysis have been performed on the proteins isolated from the maize stack itself. No data are presented that indicate that proteins have been isolated from the stack and analysed for pH or temperature stability. It is also unclear from this section what pH values that have been used for the analysis. The pH in the human digestive tract varies greatly. It ranges from 1.5 to 8.5 depending on how long time it was since food was eaten, disease state, where in the stomach the measure is made and several other issues. This can indicate that a proteolytic degradation assay should be performed over a pH range to look at stability of proteins over pH range, and also over time. It is however possible that processing, and also the matrix used for analysis, might have an impact on the digestibility of the proteins analysed by altering the "susceptibility to gastrointestinal enzymes (Takagi et al 2003). In Verhoeckx et al (2015) it is therefore suggested that a "combination of processing and digestions" should be performed in the assessment (allergenicity assessment specially mentioned) to look at impacts resulting from protein and peptide fragments in functional assays. The solubility of the proteins after these treatments is also an issue that must be considered as it might impact the results of the assays.	The GMO Panel has previously evaluated the safety of the Cry1A.105, Cry2Ab2, PMI, Vip3Aa20 and CP4 EPSPS (including its variant CP4 EPSPS L214P) proteins individually, and no concerns on allergenicity were identified in the context of the applications assessed. No new information on allergenicity of these proteins that might change the previous conclusions of the GMO Panel has become available. Based on the current knowledge, and as none of the newly expressed proteins showed allergenicity, no reasons for concerns regarding the simultaneous presence of these newly expressed proteins in this four-event stack maize affecting their allergenicity are expected. In relation to the comment on human digestion. It is noted that *in vitro* protein degradation studies were considered as additional information for the safety assessment of the newly expressed proteins. The GMO Panel has recently published (2017) a guidance document on allergenicity providing additional considerations on the *in vitro* protein degradation studies. In Annex B of this document, the 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*. In this Annex, the impact of processing and matrix was also discussed. This 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 extent to which either the intact protein or resistant fragments remain after *in vitro* digestion. However, the GMO Panel considers 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. After this period, EFSA will assess whether the test adds value to the allergenicity risk assessment and, if so, what further steps are needed for its final implementation in the form of guidance for applicants. Finally, the development of advanced methods for the allergenicity and adjuvanticity assessment of proteins is desirable. EFSA has been involved in past EU funded projects on

				the topic and is committed to incorporating latest scientific developments in its risk assessment process, when appropriate. EFSA is moving forward the field of allergenicity assessment being proactive in considering new scientific developments in the area (EFSA GMO Panel, 2017c). Future discussions involving the international scientific community could focus on building up new strategies for the allergenicity assessment
Norway	VKM	II.1.3.6 Effects of processing	The applicant should explain in detail why the processing steps involving low temperatures, such as production of high-protein maize gluten meal, does not increase the relative concentration of the transgenic proteins rather than reduce the concentrations. This may be of particular importance for the stacked event since a higher pre-processing concentration of transgenic proteins is expected than for the single events. Knowledge of protein concentrations is of importance when considering possible effects of combinations of transgenic proteins.	In the frame of animal dietary exposure to the transgenic proteins, the applicant provided estimation of their levels in maize gluten meal, applying a factor of 7.1 fold, based on the protein content of gluten meal relative to maize grain, assuming that no losses of NEP occur during processing (see Scientific opinion, section 3.4.3.5). Moreover, on the basis of the known biological functions of the individual newly expressed proteins, there is currently no expectation for possible interactions relevant to the food and feed safety of the four-event stack maize (see Scientific opinion, section 3.4.3.3). Therefore, according to Regulation (EU) No 503/2013, no further information on possible effects due to the combination of these transgenic proteins is needed.
Norway	VKM	II.1.4.1 Testing of newly expressed proteins	2. The VKM GMO Panel does not find that the following claim made by the applicant is substantiated: "CP4 EPSPS, Cry1A.105, Cry2Ab2, Vip3Aa20 and PMI proteins have no synergistic or antagonistic effects to each other. Their modes of action and sites of biological activity are different and there is no known or conceivable mechanism of interaction between CP4 EPSPS, Cry1.A105, Cry2Ab2, Vip3Aa20 and PMI which could lead to adverse health effects in animals or humans." This statement should be justified by evidence-based data or followed up by appropriate studies in accordance with the EFSA guidelines (2011). The applicant has not provided data that exclude possible combined effects of the newly expressed proteins in the stacked event. Different modes of action do not necessarily prevent interaction.	The GMO Panel took note of the comment. As regards interactions among newly expressed proteins, the GMO Panel is of the opinion that there is currently no expectation for possible interactions among these which are relevant to the food and feed safety of the four-event stack maize considering the known biological function of the individual newly expressed proteins. Therefore, no additional studies on these proteins, individually or in combination, are considered necessary by the GMO Panel. Please see Section 3.4.3.3 of the Scientific Opinion for further details.
Norway	VKM	II.1.5.1 Assessment of allergenicity of the newly	Most immunologic adjuvant experiments on Cry –proteins have been performed on Cry1Ac, and some of these studies have indicated adjuvant properties (VKM, 2012). To our knowledge the Vip3Aa20, Cry1A.105 and Cry2Ab2 proteins have not been studied experimentally for potential adjuvant	The GMO Panel assessed the safety of the newly expressed proteins following its guidance documents which are in line with internationally agreed standards. For the assessment of allergenicity and adjuvanticity, please see section 3.4.3.4 of the scientific opinion on this GM maize. The development of

		expressed protein	properties. Although these proteins do not show sequence resemblance to known adjuvants like cholera toxin and E. coli heat-labile enterotoxin (as referred to by the applicant in Brunner et al., 2010 and Reed et al., 2008), The VKM GMO Panel therefore highlight the need for further clarification on the potential role of these proteins as adjuvants as part of the risk assessment. This may be of particular importance for high-protein fractions, e.g. maize gluten meal, produced under low temperatures, since levels of the transgenic proteins could be up-concentrated in these fractions. The VKM GMO Panel considers that the referred experimental data from the single events alone do not sufficiently answer uncertainties related to the combined exposure of the transgenic proteins, e.g. from protein isolates from the stacked event, and requests that the applicant provide experimental data to exclude adjuvant properties.	advanced methods for the allergenicity and adjuvanticity assessment of proteins is desirable. EFSA has been involved in past EU funded projects on the topic and is committed to incorporating latest scientific developments in its risk assessment process, when appropriate. EFSA is moving forward the field of allergenicity assessment being proactive in considering new scientific developments in the area (EFSA GMO Panel, 2017c). Future discussions involving Member States and the international scientific community at large could focus on building up new strategies for the allergenicity assessment. Additional considerations on the topic and in particular on adjuvanticity of Cry proteins can be found in recent EFSA publications (EFSA, 2018b, 2019).
Spain	MAGRAMA (CNB)	II.1.4 Toxicology	Comments to the notification EFSA-GMO-NL-2016-131, for the import, processing and use as food and feed of genetically modified maize MON 87427 x MON 89034 x MIR162 x NK603 from Monsanto Company. The proteins expressed in this maize are CP4 EPSPS, Cry 1A.105, Cry2Ab2, Vip3Aa20 and PMI. The dossier states that these proteins have been shown, based on molecular characterization, safe use, lack of similarity to known toxic proteins and rapid digestion in simulated gastric fluid. 90-day studies with unique events showed no toxicologically significant effects. However, from our point of view, and in order to assess in a more complete way the safety of this stack event, it would be desirable to have a 90-day study with complete food. The dossier also refers, as justification for the safety of this GMO, the high margin of exposure (MOE) obtained from the acute toxicity study. From our point of view this approach is completely wrong, because not only the acute toxicity studies provide little value to the risk assessment for repeated consumption of food or feed derived from genetically modified plants, but also because the NOAEL to be used for carrying out risk characterization and establish a MOE, has to be a NOAEL derived from a repeated dose toxicity study, not derived from an acute toxicity study.	The GMO panel took note of the comment and agrees on the considerations on the not adequate use of MoE obtained from acute toxicity studies. However, the GMO Panel considers that a 90-day study on the whole food/feed from the four-event stack maize is not necessary based on the outcome of the molecular characterisation assessment, comparative analysis and toxicological assessment, and the lack of indication of findings relevant to food/feed safety related to he stability and expression of the inserts or to interaction between the transformation events, and of modifications of toxicological concern in the composition of the four-event stack maize. Therefore, in line with EFSA GMO Panel (2011a) and Regulation (EU) 503/2013, in the absence of a hypothesis animal studies on food/feed derived from the four-event stack maize are not necessary.

			Therefore, as discussed, it would be desirable to have a 90-day study with complete food.	

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 below.

EFSA (European Food Safety Authority), Fernandez Dumont A, Lanzoni A, Waigmann E and Paoletti C, 2018b. Relevance of new scientific information (Santos-Vigil et al., 2018) in relation to the risk assessment of genetically modified crops with Cry1Ac. EFSA supporting publication 2018:EN-1504. 13pp.

EFSA (European Food Safety Authority), 2019. Scientific advice on the internal review under Regulation (EC) No 1367/2006 of the Commission's decision authorising the placing on the market of genetically modified maize MON 87427 × MON 89034 × 1507 × MON 88017 × 59122 and subcombinations. EFSA supporting publication 2019:EN-1603. 25pp.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messéan A, Nielsen EE, Nogué F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017c. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp.