Application EFSA-GMO-UK-2005-21 (Maize 59122x1507xNK603) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry of Health, Family and Youth	General comments	Detection method As long as no official (guidance) document on the interpretation of detection results of the described method for stacked events are available, no approval for placing on the market of this product should be given.	Not in the remit of the Panel.
Austria	Ministry of Health, Family and Youth	General comments	Post-market monitoring of GM-food According to Art. 5 (3) k) of EU-Regulation 1829/2003 a post-market monitoring-plan should be added to the dossier.	Since no changes have been identified in the composition and the nutritional value of the hybrid maize 59122 x 1507 x NK603 and since it is unlikely that the intake will be different from that of conventional maize, the Panel is of the opinion that no post-market monitoring of GM food/feed is necessary.
Austria	Ministry of Health, Family and Youth	General comments	Concerning all single events of this notification, Austria is still of the opinion that their risk assessment with regard to e.g. molecular characterisation, allergological and toxicological as well as environmental risk assessment can not be regarded as sufficient. Due to these lacks in the presented scientific data of the single events, it is not regarded as apropriate to apply for approval of the multi-stacked event before clarifying the shortcomings of the single events.	The single events (59122, 1507 and NK603) as well as the double stacked events 59122 x NK603 and 1507 x NK603 have been the subjects of earlier assessments and have received EFSA GMO Panel scientific opinions (EFSA, 2003a, 2003b, 2004a, 2005a, 2005b, 2006c, 2007b, 2008). Maize 59122 was authorised under Regulation (EC) No 1829/2003 with Commission Decision 2007/702/EC (EC, 2007). Maize 1507 was authorised under Directive 2001/18/EC by Commission Decision 2005/772/EC (EC, 2005b) for feed use, import and processing. The placing of 1507 maize on the market for food use received authorisation under Regulation 1829/2003 with Commission Decision 2006/197/EC (EC, 2006). Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004). The use of food and food ingredients from NK603 maize was authorised under the Regulation (EC) No 258/97 (EC, 1997) by Commission Decision 2005/448/EC (EC, 2005a).
Austria	Ministry of Health, Family and Youth	C. Information relating to the genetic modification	he data submitted to conclude the molecular equivalence of GM Maize 59122x1507xNK603 with the parental GM lines (59122, 1507 and NK603) consist of Southern Blots to demonstrate presence of the introduced traits (Cry34Ab1, Cry35Ab1, Cry1F, Pat and EPSPS) in GM Maize 59122x1507xNK603.	Additional info has been requested on the intactness of the inserts and the flanks. Molecular equivalence of the 59122, 1507 and NK603 insert in the hybrid line was determined by Southern analysis, using *Sac*I, *Hind*III and *EcoRV* digested

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			However the used probes do not span the complete inserts introduced into the parental GM plants used to construct GM Maize 59122x1507xNK603. They only represent parts of the coding regions of the introduced genes. The rather limited scope of analysis as presented does not result in a comprehensive examination of the inserts present in GM Maize 59122x1507xNK603. Some of the data are furthermore not fully conclusive. • Some fragments are not clearly visible due to faint hybridisation with controls (e.g. fig. 7: SacI digests and PHP17662 plasmid DNA hybridised with Cry24Ab1 probe), or minimal overlap of probes with the DNA fragments to be detected (fig 8: SacI digests hybridised with Cry35Ab1 probes; fig 9: HindIII digest hybridised with Cry1F probe). • Some fragments which are expected to be identified by the probes used cannot be distinguished due to imperfect separation of fragments with comparable molecular weights (e.g. fig 8, fig 9). Therefore it is not possible to assess whether the expected molecular composition is verified for GM Maize 59122x1507xNK603. • Other fragments, e.g the 4,1 kb fragment expected as hybridisation signal in Southern Blots of HindIII digested DNA from GM Maize 1507 and GM Maize 59122x1507xNK603 are not detected for the GM Maize 1507 control strain (fig 10). This is attributed by the notifier to “variable hybridisation” of the used pat-probe. However to obtain unequivocal results probes should be used which do not show such “variable” patterns of hybridisation. • Some parts of the analysis, e.g. the demonstration of the molecular identity of the NK603 trait of GM Maize 59122x1507xNK603 are limited to internal insert sequences and do not cover the border regions of the insert. For a complete molecular characterisation and the comparison with parental GMO-strains, results for insert and border regions of the introduced traits should be presented by the notifier. The method used for all Southern experiments employed Digoxygenin-	genomic DNA and probes of the *pat*, *Cry1F*, *Cry34Ab1*, *Cry35Ab1* and *cp4 epsps* genes. From the hybridisation patters of 59122x1507xNK603 and all parental lines it was concluded that the organisation of sequences in the insert are unchanged. Also the intactness of the 1507 insert and of the 3’ side of the 59122 was confirmed. Additional information has been supplied on the intactness of the NK603 insert and the 5’ of the 59122 insert in the hybrid line. The intactness of the NK603 insert was demonstrated by Southern analysis of *Msc*I and *Sca*I-digested DNA. The intactness 59122 insert in the hybrid line was confirmed by results obtained by event-specific real time PCR of the 5’ region of the 59122 insert. An explanation has been requested on the results of fig 8, 9, 10 and 11. The applicant supplied explanations for the observed results. In addition a new Southern analysis of *Sac*I digested DNA, probed with *cry*35AB1, was supplied. The GMO Panel is of the opinion that the explanation and new blot are satisfactory and that the results of the blots do not pose a safety concern. The DIG labelled MW markers migrate slower in agarose gel. However since hybridizing fragments

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			labelled probes and DNA Molecular Weight Markers (MWM). As noted in the application these DIG-labelled MWM typically migrate slower in Agarose gels than expected from their molecular weight by a margin of 5-10%. This makes the necessary comparisons difficult and adds to the methodological difficulties of a method like Southern blotting. The notifier therefore has to assume that the identified fragments are of the expected size rather than unequivocally demonstrating it. We therefore suggest that methods are used for molecular characterisation, which do not introduce avoidable uncertainties. The notifier states that a Non-GM Maize is used as control for the molecular characterisation of GM Maize 59122x1507xNK603. However, the necessary information to verify this assertion is missing in the dossier and the respective annexes. The controls therefore need to be described in an adequate way. For a detailed characterisation of modifications present in GM Maize 59122x1507xNK603 the notifier makes reference to the data submitted for parental GM events. However since the demonstration of molecular identity lacks strength due to the indicated inconsistencies, we do not regard the conclusions of the notifier justified.	migrate equivalently with the hybridizing bands of the plasmid controls, the GMO Panel is of the opinion that the size of the fragments is sufficiently demonstrated. For the analyses of the integrity of the inserts in the stack, the only meaningful comparators are the single events.
Austria	Ministry of Health, Family and Youth	C. Information relating to the genetic modification	The referenced data for parental GM events itself were criticised in our comments addressed to the respective applications. The mentioned concerns still prevail because similar data are submitted in the application for GM Maize 59122x1507xNK603: Concerning GM Maize 59122: Regarding characterisation of the maize genomic regions at the border regions 5´and 3´of the transgenic insert the annexed laboratory study report (Annex 7 of the technical dossier) states that "No further identification of the maize genomic border sequences was possible due to limited sequence homology with publicly available sequences in GenBank." (Annex 7, p.3). This conclusion was drawn upon a homology search against sequences contained in GenBank Rel.	An updated bioinformatic analysis has been requested on all three events. For 59122 an updated BLAST analysis indicated that the DNA in 59122 was inserted 1032 bp downstream of the coding region of a maize pentatricopeptide repeat (PPR) protein, the empty pericarp4 (*emp4*). This PPR protein is essential for seed development in maize. In event 59122 seed development is not affected suggesting that expression of *emp4* was not altered by the insertion. For 1507 an updated BLAST analysis of the flanking DNA sequences suggests that the insert in 1507 is flanked by a putative RIRE2 retrotransposon (downstream) and a Huck1 retrotransposable element (upstream).

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			138 (Oct. 25th 2003). It is notable however that since the time this study was undertaken the number of DNA sequences stored in GenBank significantly increased (for a graphic representation see: http://www.ncbi.nlm.nih.gov/Genbank/index.html). It is therefore necessary to compare the identified border sequences in GM maize 59122 against a current version of the database to aid better identification of the genomic region into which the transgenic DNA was inserted. Therefore the submitted information is incomplete. Concerning GM Maize 1507: Incompleteness of the molecular characterisation was criticised for various applications of GM Maize 1507, like the referenced application (EFSA-GMO-NL-2004-02), specifically concerning location and size of the additional copy of cry1F in the GM Maize 1507 genome. Further concerns address the question whether this additional copy of the cry1F gene in the GM event 1507 contains an ubiquitin promoter region. Concerning GM Maize NK603: With regard to data submitted with preceding applications for GM Maize NK603 some inconsistencies were criticised, specifically concerning a putative 3´splice site that is found in the 3´genomic region flanking the insert in GM Maize NK603. This splice site could result in the expression of putative fusion proteins, which would be larger than anticipated by the notifier.	For 1507 the updated bioinformatic analysis confirmed the location of the additional copy of the *cry* gene in the insert. Analysis of novel ORFs that have the potential to be transcribed, do not give rise to proteins that have significant homology to know toxins or allergens. Therefore the GMO panel concludes there is no safety concern. The Panel requested an updated bioinformatic analysis on the flanking regions of event NK603. The updated bioinformatic analysis raised no safety concerns.
Austria	Ministry of Health, Family and Youth	D, 03 Information on the expression of the insert	The control used is described as a hybrid from crossing non-GM Maize 38P05 (Pioneer: 1W2X61B genetic background) with null segregating offspring from a GM Maize 59122 line (05F background). According to EFSA guidance Non-GMO controls should not be derived from a genetically modified strain. We therefore regard the used control strain as	Additional information has been requested on the controls used for protein expression and was supplied by the applicant. The applicant provided new data from European field trials with 59122x1507xNK603 in 2005, on three locations in Spain. In these trials all three parental lines were used as controls. Results on expression levels of

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			inadequate, given that a non-modified strain with a comparable 1W2X61Bx05F genetic background should have been available. The controls used furthermore do not meet the requirements of the newly published EFSA "Guidance Document of the Scientific Panel on GMOs for the risk assessment of genetically modified plants containing stacked transformation events" [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.], calling for testing of a Non-GMO control strain of comparable genetic background together with the parental GM-events and the Stacked Event. No expression levels from untreated GM Maize 59122x1507xNK603 are presented in the application. Data are submitted in the technical dossier as pooled values over all locations (5 USA and 1 Canada). The results show that the expression levels vary considerably across locations analysed. We therefore regard the analysis as incomplete and insufficient. In our opinion results of appropriate controls need to be used and additional data from untreated GM Maize 59122x1507xNK603 should be submitted. These data should be analysed and presented for individual sites and across sites.	the three Cry proteins, the PAT and the CP4 EPSPS protein demonstrated levels in the stacked line to be in the same range as in the parental lines. No effect was apparent for herbicide application. The GMO panel considers these data on expression of the inserts sufficient for the safety assessment. The grain of the hybrid that will be imported is treated with herbicides. Taking into account that all single events were previously assessed positively, expression levels of treated plants is considered to be appropriate.
Austria	Ministry of Health, Family and Youth	D, 03 Information on the expression of the insert	Expression of potential fusion proteins Except for GM Maize 59122 no updated analysis on expression of potential fusion proteins was submitted. The data on GM Maize 59122 were reported in August 2005 and contain an analysis of open reading frames at border sequences of the analysed insert and sequence comparisons to assess any potential toxic or allergenic characteristics of potentially expressed fusion proteins. However in silico analyses are not supported by experimental data to assess which potential fusion proteins are actually transcribed in GM maize 59122x1507xNK603. Such data are necessary to substantiate the conclusions of the	An updated bioinformatic analysis has been requested for both events and was supplied by the applicant. This analysis confirms earlier safety assessments of both events. Bioinformatic analyses indicate that should any of the transcripts be translated, none of the potential peptides would show homology with known allergens, toxins or other biologically active proteins or peptides.

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			notifier.	
Austria	Ministry of Health, Family and Youth	D, 04 Information on how the GM plant differs from the recipient plant in: …	The control used is described as a hybrid from crossing non-GM Maize 38P05 (Pioneer: 1W2X61B genetic background) with null segregating offspring from a GM Maize 59122 line (05F background). According to EFSA guidance non-GMO controls should not be derived from a genetically modified strain. We therefore regard the used control strain as inadequate and request submission of data acquired according to existing guidelines. Furthermore parameters "insect damage" and "disease incidence" were evaluated only semi-quantitatively and the notifier did not differentiate between individual insect pest species or diseases. Such an assessment can only indicate rough differences in the susceptibility of a plant to certain stressors. Also certain differences in the susceptibility to specific insects (e.g. secondary pests) or diseases cannot be detected by such an analysis. More specific data are requested to justify the conclusions by the notifier.	On the request of the GMO Panel, the applicant provided information on the breeding scheme of the comparators. Field trials using the non-GM comparator were carried out in 2004. The GMO Panel based the comparative assessment of 59122 x 1507 x NK603 maize primarily on the European field trials which had used non-GM maize as comparator. See also section 4.1.2 The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x NK603 and does not include cultivation. Considering the proposed uses of maize 59122 x 1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment.
Austria	Ministry of Health, Family and Youth	D, 07.01 Comparative assessment	(See also Figures 2 and 3 as attachment – all tables and figures can only be transmitted via mail due to the technical inability via EFSA-Net. Nevertheless Austria kindly asks the GMO-Panel to take them into consideration.) The main purpose of establishing substantial equivalence is to prove, that the GM had no effect on the chemical composition of the test line. For this purpose field trials of the GM corn and a genetically close control line are conducted to provide comparable growing conditions such as soil type and weather to exclude as far as possible other	1.Given that the single events have been evaluated and found to be safe, the GMO Panel considers that one season of field trials is sufficient to demonstrate the compositional equivalence of the GM plant containing stacked events with its comparators (see references, EFSA 2007) 2. The GMO Panel considered the fact that treatment of the single events with corresponding target herbicides did not affect their agronomic / compositional characteristics compared to untreated plants. Therefore the GMO Panel accepted the design of the field trials although plots untreated with both target

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			influencing factors. All significant analytical differences within locations can than be evaluated as to their biological significance and consistency. It is therefore futile to access historical data collections, where different growing and weather conditions as well as different cultivars and cultivation methods obfuscate scientifically sound conclusions. Significant differences have to be discussed as to their functional importance as well as to other possible causes (e. g. nutritional conditions of the soil). The variability of agronomic differences between the locations is never considered. Based on such data the notifier concludes that GM Maize 59122x1507xNK603 is substantially equivalent to non-modified maize in spite of differences observed across locations and on a per location basis. It is therefore unclear, which significant differences would actually trigger further investigations. The observed differences should gain more attention to clarify the underlying cause as the assessment of compositional equivalence between the GM and the non-GM plant is not considered to be a safety assessment in itself, but rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart (Codex Alimentarius Commission 2003 [Codex Alimentarius Commission (2003). Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45.]). The following procedures for future field trials and compound comparisons seem advisable: 1. field trials should be conducted in more than one season 2. an unsprayed control of the test GM variant should be included to distinguish between GM- and treatment-related differences 3. more emphasis should be put on the choice of the respective non-GM control maize, especially where stacked events are concerned 4. the variability of the cultivation sites including soil samples should be defined to establish an interpretation background for significant differences 5. the main focus should be on	herbicides had not been systematically included in the studies. 3. The pedigree information provided by the applicant for the non-GM control maize showed that the control represented an appropriate comparator for 59122xNK603 maize in the field trials. By taking into account the additional information provided by the applicant, the Panel accepted the comparative compositional analysis described in the application. The Panel was satisfied with the selection of field trial sites being representative of the various environments in which the GM plants will be cultivated. Soil samples were not required as an interpretation background for significant differences. The Panel confirms that levels of maize 59122xNK603 constituents were compared to the respective levels observed for appropriate comparators. Relations between compounds were sufficiently addressed with a particular focus on the observed compositional differences (protein and amino acid levels, see text in the opinion). Based on the results of comparative analysis it is concluded that 59122x1507xNK603 maize is compositionally and agronomically equivalent to conventional maize, except for the presence of Cry34Ab1, Cry35Ab1, Cry iF, CP4EPSPS and PAT proteins in 59122xNK603 maize. Therefore, no follow-up on compositional differences is required.

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			the comparators, not on reported data and reference lines, since the GM influence is being investigated, not general quality standards 6. more weight should be put on relations between compounds since they are more meaningful in biological systems 7. unexplained differences should be followed up, either by literature discussions or new tests 8. molecular characterisation: via sequencing only the gross structure of the insert could be visualized. Point mutations, small deletions and rearrangements might occur during breeding. Structural modifications of the insert are not detected through Southern blot, these structural modifications could occur during traditional breeding and might impose a risk 9. the stability of the insert over several generations should be evaluated and only hybrids with stable inserts should be used to produce seed.	Following a request from EFSA the applicant has provided an up-to-date bioinformatic analysis of the transgenic locus including flanking regions. A detailed description of this analysis can be found in section 3.1.2. of the GMO Panel opinion. Based on the analyses provided the putative ORF amino acid sequences identified from maize 59122x1507xNK603 do not present any significant sequence identity with known toxins and allergens or with maize genes of known function. The molecular data supplied by the applicant do not suggest a structural modification due to the conventional breeding of the single events. The stability of the single events was determined over several generations, stability of the stacked event over one generation. This is considered to be sufficient from a safety point of view. The agronomic characteristics of 59122x1507xNK603 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns.
Austria	Ministry of Health, Family and Youth	D, 07.01 Comparative assessment	The control used is described as a hybrid from crossing non-GM Maize 38P05 (Pioneer: 1W2X61B genetic background) with null segregating offspring from a GM Maize 59122 line (05F background). According to EFSA guidance non-GMO controls should not be derived from a genetically modified strain. We therefore regard the used control strain as inadequate and request submission of data acquired according to existing guidelines. The results of the compositional analyses according to OECD guidelines show significant differences for 11 analytes across locations (see technical dossier p. 28 ff, and Annex 6). Significant differences were found for analytes of	Field trials using the non-GM comparator were carried out in 2004. The GMO Panel based the comparative assessment of 59122 x 1507 x NK603 maize primarily on the European field trials which had used non-GM maize as comparator. See also section 4.1.2 The GMO Panel requested from the applicant further information with respect to the compositional analysis data and in particular the statistically significant differences observed. The GMO Panel concluded that expression of the newly introduced genes in 59122 x 1507 x NK603 maize did not result in any effect on the chemical composition and that 59122 x 1507 x NK603 maize is compositionally equivalent to its non GM

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			most of the categories of compounds (7 out of 8 categories). A substantial number of analytes furthermore showed significant differences at one or two out of six individual locations (8 out of 11 parameters with significant differences across locations). No data were submitted for untreated GM Maize 59122x1507xNK603. However data from literature other than OECD documents were used to calculate a range for additional comparisons and in case significant differences between the GM and the non-GM maize were observed. These data are derived from different sources and from field trials other than those carried out specifically with GM Maize 59122x1507xNK603 and the control line. This approach results in data derived from maize plants grown under different conditions and in different years and introduces additional variation which may obscure relevant differences. All significant differences were levelled by the sentence: "In addition the across location mean values in the test and control hybrids were within the reported literature ranges." Since a detailed data review is given in the chapters on the five GM maize lines under investigation here, only principal observations are mentioned. Comparing all five compositional data sets has been useful in detecting the common trend, that crude protein levels are higher in the GM lines and carbohydrate contents lower as compared to their respective controls, indicating a shift between the two main plant metabolic cycles (C and N) in favour of the N-cycle (Table 9; Fig. 2 forage +3 grain). Unfortunately the C/N relations were not measured. This means a change of the plant´s physiological state (e.g. resistance and susceptibility to pathogens) and influences the metabolic performance accordingly. These results were of course also greatly dependent on the control line used. The calculations for table 9 were done by using the available data: carbohydrates % DW and crude protein % DW to get at least a clearer picture of the	counterpart and conventional maize except for the presence of Cry34Ab1, Cry35Ab1, Cry1F, CP4 EPSPS, CP4 EPSPS L214P and PAT proteins (section 4.1.3)

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			differences described.	
Austria	Ministry of Health, Family and Youth	D, 07.04 Agronomic traits	Concerning the stacked event: Agronomic characteristics were presented in the dossier as numbers across sites (technical dossier p. 84, table 9). A calculation across sites masks differences at single sites due to regionally different frequencies of pest or pathogen infestations. The individual parameters should therefore also be analysed on a single location basis for all data (USA/Canada). There was significantly more phosphorous in the forage samples of the GM stacked event, corroborating the findings in GM maize 59122xNK603. There are 51 significant differences, but they are not consistent over all locations (Table 8). The tendency of lower protein levels combined with higher carbohydrate contents as found in the GM maize 59122xNK603 is not corroborated by this field trial. Only one location MO1 has similar values. All significantly higher levels of amino acids on the location MO1 are also reflected in a higher level of crude protein (8,82 vs 7,55 % DW not sign.) and a lower level of carbohydrate (83,8 vs 84, 2 % DW not sign.). But the mean levels of carbohydrates were again significantly lower in the compared GM grain. The differences between sites should be explained and discussed. This field experiment and the one with GM 59122xNK603 was conducted in the same region and the same harvest year. The control maize forage of the GM 59122xNK603 had lower protein concentration than both stacked events (7,33 vs 8,72 and 8. 98 % DW), whereas the carbohydrate levels were comparable in the trial with 59122x1507xNK603, they were significantly lower in 59122xNK603 as compared to its control. It would be necessary to compare the stacked events as well as their respective controls to come to a final conclusion. Furthermore it is not clear what triggers the choice of control line. In the case of 59122xNK603 a commercial hybrid was used, whereas the comparator for 59122x1507xNK603 was a near-isoline F1 hybrid. Table 8: Results from field	Based on the results of comparative analysis it is concluded that 59122 x 1507 x NK603 maize is compositionally and agronomically equivalent to its non GM counterpart and conventional maize, except for the presence of Cry34Ab1, Cry35Ab1, Cry1F, CP4 EPSPS, CP4 EPSPS L214P and PAT proteins in 59122 x 1507 x NK603 maize. Based on the assessment of data available, including the additional information provided by the applicant in response to the Panel request, for 59122 x 1507 x NK603 maize, for 59122xNK603 maize, for 1507 x 59122 maize, for the single events and for appropriate non-GM controls, the GMO Panel has found no indication that crossing of 1507, 59122 and NK603 maize results in an interaction of the newly expressed proteins which causes compositional or agronomic changes.

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			trials in 6 locations in USA and CA (2003):(please see comments transmitted via e-mail) In the field comparisons with the stacked event 59122x1507xNK603 (USA, CA) the germination success (number of plants from 60 seeds) was significantly lower in two of the six planting sites and the final plant population was lower on four sites, once even significantly. These results could indicate that the agricultural performance of the stacked event 59122x1507xNK603 does not point to higher yields, the main reason for designing GM plants.	
Austria	Ministry of Health, Family and Youth	D, 07.04 Agronomic traits	Concerning GM maize NK603 All compared agronomic traits and compositional values of the test GM maize NK603 and the control line were reported to be similar. Field trials in the USA (1998) and Europe (1999) showed no difference in agronomic traits between the test and control maize. Regarding substantial equivalence of the grain small but significant differences were found in the amino acids arginine, cystine and phenylalanine, for the minerals Ca, Mg and P as well as in the fatty acids palmitic, stearic, oleic and eicosanoic acids. The forage samples showed small but significant differences concerning carbohydrate, protein and moisture contents in one of three field trials. Moisture is not a key nutrient, but depends on harvest time. Basically in comparative field trials the test corn samples should be harvested on the same day, since moisture may influence other traits such as dry matter content and storability before drying. Furthermore carbohydrates and proteins are basic key nutrients and showed significant differences in forage as well as in grain samples. Similarly to 59122 and 1507 crude protein contents were increased and carbohydrates decreased in the GM test variants. But since all levels were within the reported literature range these differences were not considered treatment-related and were not discussed in connection with other possible causes for the differences such as differentiating properties of the	The single event NK603 and newly expressed protein in single events have been already assessed by the GMO Panel. Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004a). The use of food and food ingredients from NK603 maize was authorised under the Regulation (EC) No 258/97 by Commission Decision 2005/448/EC (EC, 2005).

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			cultivation sites.	
Austria	Ministry of Health, Family and Youth	D, 07.04 Agronomic traits	General remark Although a wide range of biocides was applied in the field trials no residue levels are mentioned in the dossier. This could be of interest since some of these biocides have already been banned in the EU or are being re-evaluated in the current EU-wide pesticides peer review process. A pesticide no longer registered in the EU may still be used outside the EU, if it is registered for use in the country where the crop is grown, providing that no detectable residues of that pesticide are left on the crop and as long as the EU customer has approved of the use of that pesticide on the imported crop supplied for sale. In addition to the site-dependent application of pesticides (e.g. Atrazine, Chlopyriphos, Dimethanamid, Terbufos, Metolachlor, Permethrin, Tefluthrin, Dicamba, Alachlor, Bifenthrin, triazole fungicides, Carbofuran, Perrethrin, Cyperrethrin) the anticipated multiple use of the active ingredients glyphosate and glufosinate-ammonium on stacked crops with tolerance resp. resistance to both broad spectrum herbicides could entail higher levels of residue cocktails. Risk confounding effects such as synergisms between residues and novel proteins or new metabolites resulting from herbicide inactivation (e.g. PAT protein/glufosinate-ammonium) or interactions between novel proteins in stacked events are not addressed. Risk assessment with respect to plant protection products is within the scope of Directive 91/414/EEC (EC 1991), but possible interactions are not covered. With glufosinate-ammonium spraying, for instance, the additional metabolites and degradation products of the herbicide resulting from PAT inactivation need to be monitored. To date, the likelihood of exposure and the toxicological impact of such exposure are not sufficiently clear and should be covered by regulations for herbicide use.	The overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would impact on the composition of the 59122x1507xNK603 maize or on its food/feed safety. See text in the opinion. Issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the GMO Panel.
Austria	Ministry	D, 07.04 Agronomic	Concerning GM maize 59122: All compared	The single events (59122, 1507 and NK603) as well as

Country	Organisation	Reference	Comment	EFSA GMO Panel response
	of Health, Family and Youth	traits	agronomic traits and compositional values of the test GM maize 59122 and the control line were reported to be similar. Applied pesticides in the USA and CA trials were: Atrazine, Chlopyriphos, Dimethanamid, Terbufos, Metolachlor, Permethrin, Tefluthrin, Dicamba and in the Chile trial Alachlor, Dimethenamid, Bifenthrin, Chlorpyrifos, Flutriafol (triazole fungicides), Carbofuran (MRL EU: Maximum level 0,002mg/kg cereals), Perrethrin and Cyperrethrin. No residue levels (RLs) have been analysed and compared. Some of the pesticides (italic) are not permitted for application within the EU. 50 significant differences in the USA and CA field trials (Table 1) and 26 significant differences in the Chile field trials (Table 2) across locations and within locations are not regarded as biologically important, because these differences were not consistent in all field comparisons and are within the range of reference values. All values were within the historical range based on content analyses from 1982 and 1987 (Watson), 1988 (Wych), 1994 and 1997 (Iowa Gold Catalog), 2001 (Luna et al.), 2002 (OECD) and 2003 (ILSI – International Life Sciences Institute). The range is established by using the lowest resp. highest value obtained from the above mentioned literature (= combined ranges). No literature values were available for the sec. metabolites inositol and furfural. Since no attempt was made to explain the significant differences by referring to other composition influencing parameters such as different soil nutrient levels or cultivation methods these data remain inconclusive. The variability of the cultivation sites has to be discussed to offer alternative reasons for significantly different plant contents. Plant and soil are intrinsically linked to form one system; therefore field trials without soil data are incomplete leading to interpretation difficulties of significantly different data as a matter of course. Concerning GM maize 1507 All compared agronomic traits and compositional values of the test GM maize 1507 and the control line were	the double stacked events 59122 x NK603 and 1507 x NK603 have been the subjects of earlier assessments and have received EFSA GMO Panel scientific opinions (EFSA, 2003a, 2003b, 2004a, 2005a, 2005b, 2006c, 2007b, 2008). Maize 59122 was authorised under Regulation (EC) No 1829/2003 with Commission Decision 2007/702/EC (EC, 2007). Maize 1507 was authorised under Directive 2001/18/EC by Commission Decision 2005/772/EC (EC, 2005b) for feed use, import and processing. The placing of 1507 maize on the market for food use received authorisation under Regulation 1829/2003 with Commission Decision 2006/197/EC (EC, 2006). Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004). The use of food and food ingredients from NK603 maize was authorised under the Regulation (EC) No 258/97 (EC, 1997) by Commission Decision 2005/448/EC (EC, 2005a). Issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the GMO Panel.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			reported to be similar, regardless of herbicide treatment (!?). Field trials were conducted in Chile as well as in Italy and France 1999 (Table 3). The following pesticides were used in Chile: Cyanazine, metolaochlor, chlorpyriphos, carbofuran, flutriafol + carbofuran, cypermetrin, lambda + cyhalothrin, dicofol, atrazin and acetochlor. Glufosinate ammonium was only on the 1507 maize. The compound analyses of the grain showed several significant differences. Compared to the near genetic line the GM test maize had less fat, manganese, stearic and oleic acid, cysteine and methionine and thiamine, but more linoleic and linolenic acid, total tocopherols and potassium (K). The field trials in Europe (1999) included an unsprayed variant of 1507. Applied pesticides included metolachlor + terbuthylazine, isoxaflutole and carbofuran, glyphosate isofenphos in Italy and atrazine, alachlor, chlormephos, dimethanamid and aclonifen in France. Glufosinate ammonium on one variant of 1507 maize. Significant differences for the GM 1506 grain, sprayed and unsprayed, estimated mean values across all sites: higher protein contents, lower carbohydrate contents, more P, K and Fe, higher contents of the amino acids glycine, threonine, valine, leucine, phenylalanine, histidine, serine, alanine, glutamic acid, proline, aspartic acid, and tyrosine, but less riboflavin. The trends were the same, but were more pronounced for the sprayed variant, especially concerning more crude protein (11.73 vs 12,04 vs 10, 98 % DW) and lower carbohydrate levels (82,46 vs 81,97 vs 83,00 % DW). Residue levels were not mentioned. The 18 significant differences were not considered of biological importance since they are within the calculated data set. No other reasons for these results were offered.	
Austria	Ministry of Health, Family and	D, 07.08 Toxicology	Concerning GM maize 1507 A 90 day rat feeding study with five different diets, two containing the GM maize 1507 (33% and 11%), one with near isogenic maize and two with commercial hybrid maize has	The single events 59122, 1507 and NK603 and newly expressed protein in single events have been already assessed by the GMO Panel. Maize 59122 was authorised under Regulation 1829/2003 with

Country	Organisation	Reference	Comment	EFSA GMO Panel response
	Youth		been carried out. Twelve Cr1:CD (SD) IGS BR rats per sex and group were in the study design as proposed by the OECD test guide line 407. The results showed significantly higher feed consumption in males of the high-dose group. Furthermore haematology analyses revealed lower mean red cell count, hemoglobin and number of eosinophils only in females of the high-dose group. The clinical chemistry evaluation showed a lower level of alkaline phosphatase in males of the high dose group. Additionally the kidney weight was lower in these male rats. Mean body weight gain in male and female rats fed diets containing 33% 1507 was higher on most test days than that of rats fed the control diet, but mean body weight gains were similar over individual test day intervals. Such transient effects should not be underrated, since they do not mean that the test substance is safe in the long run. Aberrant feeding behaviour only found on a daily or weekly basis thus not presenting a consistent trend, could be triggered by an aversion to or preference of the new feed or any numbers of physiological short-term needs of the animals.	Commission Decision 2007/702/EC. Maize 1507 was authorised under Directive 2001/18/EC by Commission Decision 2004/772/EC (EC, 2005a). The placing of 1507 maize on the market for food use received authorisation under Regulation 1829/2003 with Commission Decision 2006/197/EC (EC, 2006). Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004a). The use of food and food ingredients from NK603 maize was authorised under the Regulation (EC) No 258/97 by Commission Decision 2005/448/EC (EC, 2005). The Panel is not aware of any new information that would change its opinion. Issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the GMO Panel.
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	Relevant toxicological data as required by the newly published EFSA guidance for stacked events [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.] should be submitted. For other specific comments (see chapter Nutritional Assessment). No feeding studies including one or more generations have been conducted, although it has been shown in chemical hazard assessments, that a growing organism is more susceptible to potential food risks than an adult one. There is no information on fertility parameters, lactation performance, embryonic and pub development, pub mortality. Without information on developmental parameters in connection with GM feed any risk assessment can be considered	See responses above Single events and newly expressed protein in single events have been already assessed. The Panel is not aware of any new information that would change its opinion. In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would impact on the food/feed safety

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			incomplete, since all possibly affected entities, including children have to be taken into account. So far no conclusive and final result concerning the safety of GM maize has been obtained. 90 day feeding studies with rats evaluating the GM maize lines NK603, 59122 and 1507 revealed several significant differences but the biological meaning of these data is unclear and partly controversial. Comparing these results it was noted that some blood parameters were affected inconsistently across the trials, including haematological characteristics. The significance of alterations in mean corpuscular haemoglobin (index for erythrocytes), platelet and monocyte count is not conclusive, but could hint to possible alterations in bone marrow. These samples were collected but not analysed in the 59122 and 1507 feeding studies. In addition to the cellular immune response, humoral immune response is an important defence mechanism in toxicity events. A higher level of total protein (59122 feeding study) could be attributed to an increase of the albumin, but also the (immune) globulin – fraction. Immune globulins are easily determined with ELISA technique. For further investigations the following procedures seem advisable: 1. More sensitive technologies such as the “omics”-technologies as well as Micro-Array essays should be applied. Profiling technologies representing alternatives to animal tests permit the measurement of thousands of variables simultaneously. 2. Synergistic and/or additive effects should be investigated by feeding studies as a matter of course, independently of equivalence determinations. 3. 90 day feeding studies should be obligatory in any case, although 30% of all toxicological findings are neglected due to the short time of testing. 4. Multi generations as well as RACB studies (reproductive assessment by continuous breeding) should be performed to include reproductive parameters as well as embryo and pub development, since growing organisms are more	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			sensitive to potential adverse effects. 5. In GM stacked events the expression level of the introduced insert might be different from the parental line. The amount of newly expressed protein could be potentially toxic. 6. Potential interactions of the newly introduced genes, regulatory sequences and proteins with the host genome should be examined. Genotoxicity testing should be performed to screen for point mutations, chromosomal aberrations and DNA damage.	
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	For the whole food/feed safety assessments the applicant refers to the testing of whole grains of GM Maize 59122x1507xNK603 in a feeding study with poultry (42 day study). The control strain (091) used in the poultry feeding study is poorly described and no breeding history is given (technical dossier, Annex 12). No reference is made as to whether the same inadequate control strain as described in Annex 6 was used. Whole feed conversion studies are conducted to investigate whole feed effects on farm animals, mostly broilers, and thus reflect realistic conditions. But no general statements about potential adverse effects on the long run are possible (e.g. only 42 days in broilers). Organisms generally have the capacity to bear up with a relatively short-time exposure to inadequate feed. Furthermore the test parameters are usually limited to mortality rates, weight gain and organ weights. There is no follow up investigation or discussion of significant differences that do occasionally occur (e.g. kidney or liver weights). In any case information is missing, whether the control was derived from a previously genetically modified strain. Without this information it cannot be assessed whether the study was done in line with relevant guidance or not. In case the guidance was not followed, submission of data according to published guidance is requested. Additionally the study was performed with the differently treated GM stacked event 59122x1507xNK603 and a non-GM control. The treatments were glyphosate alone,	With regard to the safety assessment of the single events, see responses above. The Panel agrees with the comment that the broiler feeding study does not constitute a toxicological study. The applicant has provided a nutritional study on broilers using the triple stacked event 59122 x 1507 x NK603 maize as test material. The Panel is of the opinion that since 59122x1507xNK603 maize is compositionally and agronomically equivalent to conventional maize and the possibility of interactions between the expressed proteins was not identified, no toxicological or nutritional feeding studies are required to conclude on the safety of 59122x1507xNK603 maize. and considers that the feeding study provides further confirms this conclusion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			glufosinolate ammonium alone or both herbicdes combined. The kidney weight of the females fed with the combined treatment GM maize was significantly lower, an effect not found in the other diet groups. Therefore it can be deducted, that the use of both herbicides influenced the weight difference in combination with the GM. The abdominal fat yield of the GM fed males was significantly lower, irrespective of herbicide treatment. Females of the glyphosate treated GM maize diet had significantly lower carcass yield, but significantly higher thigh yield. The soy beans added to the diet contained concentrations of the CP4 EPSPS up to 0,083%. A more thorough investigation of potential adverse effects could have been expected, taking into account that so far no conclusive results have been published and the safety of GM plants is still controversially discussed by scientists but also the public. This difference is assumed to be of no relevance by the notifier based on a tolerance interval established by using three other commercial maize varieties. Furthermore, the poultry study used to assess toxicological safety constitutes a feed conversion study rather than a toxicological study. For safety considerations toxicological endpoints must be assessed rather than performance parameters as done in the chicken study supplied. Such a feeding study with chicken broilers is therefore not appropriate to assess the toxicological safety of GM Maize 59122x1507xNK603.	
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	For the toxicological assessment of GM Maize 59122x1507xNK603 the applicant refers to the assessment of the individual gene products with reference to acute toxicity studies of microbially produced test proteins among others. However some proteins (Cry35Ab1 produced by the P. fluorescens strain MR1256) show minor differences to Cry35Ab1 protein as expressed in GM Maize 59122x1507xNK603. Tests employing heterologous test proteins should be done with similar test material to obtain conclusive results. Oral toxicity studies and	See responses above Single events and newly expressed protein in single events have been already assessed. The Panel is not aware of any new information that would change its opinion. In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would impact on the food/feed safety

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			repeated oral toxicity tests were conducted with the recombinant proteins produced in bacterial expression systems to define the acute LD50 (Lethal Dosis) value. No safety concerns were detected in very high amounts administered. The extrapolation of the obtained data to a general conclusion of safety is limited, since neither pleiotropic and/or synergistic effects within organisms nor long-term effects are included. Additionally, the safety of the proteins is concluded with reference to digestion patterns of the individual proteins in simulated gastric fluids. The respective proteins were tested separately in these experiments, whereas in vivo the proteins are all present in the digestive system. Therefore the experimental setup does not reflect the real exposition scenario of consumption of these proteins in GM Maize 59122x1507xNK603. The in vitro digestibility test is very valuable for the observation of biochemical properties of the novel protein such as enzyme resistance, but seems an unsafe model for risk prediction since it lacks absolute comparability with living systems. In vivo the recombinant elements are protected within the plant tissue. The acidity (pH) of the gastrointestinal tract (GIT) is influenced by the type of diet and represents a mutual relationship with populations and metabolic characteristics of the gastrointestinal bacteria, which again are influenced by diets. Furthermore the pepsin: substrate ratio is difficult to simulate. Thus pepsin-mediated digestion is a first step in evaluating the potential allergenicity of the novel proteins and not suited to eliminate any possibility of allergenic potential. Generally, little significance can be attributed to toxicological tests with isolated gene products. This has already been mentioned by many authors (Spök et al. 2005 , Millstone 1999 , Walker 2000 [Spök A., Hofer H., Lehner P., Valenta R., Stirn S. Gaugitsch H. (2005). Risk Assessment of GMO Products in the European Union. Umweltbundesamt Wien, Band 253. Millstone E. (1999). Beyond	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			substantial equivalence. Nature 401 (6753): 525-526 Walker R. (2000). Joint FAO/WHO Expert consultation on foods derived from Biotechnology. 29 May-2 June 2000. Geneva.]) due to the fact that pleiotropic effects in the plant as well as differences in protein quality remain unconsidered. There is scientific evidence that the parameters studied do not necessarily prove the toxicological or allergological safety of proteins (see references in Spök et al., 2005). No data on potential interactions of introduced traits with relevance to adverse effects are given. Such an assessment is crucial for an assessment of Stacked Event GMOs according to published guidelines [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.] and therefore requested.	
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	Despite the above mentioned significant differences concerning hematology, clinical chemistry, urine composition, body weights and feed consumption it is concluded that NK603 is equivalent to its near isogenic maize line. The differences are defined as random occurrences and of no biological significance, since they mostly lie within the range of biological variance. But the main focus here is on the comparison between NK603 and its near genetic line to investigate GM-related influences possibly even in connection with herbicide treatments, not on the possibility to integrate the findings within a range of data from other feeding studies, thus levelling the differences between the two main study groups. This is only argumentative but no final proof that the significant effects are of no importance. The results should rather be compared with other GM risk feeding studies to compare and possibly crystallise common features. According to Seralinì the statistical analyses should include standard multivariant methods like principal composant analysis (PCA), Data Mining, Manova, to avoid a risk of neglecting effects which	See responses above Single events and newly expressed protein in single events have been already assessed. The Panel is not aware of any new information that would change its opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			could be of biological relevance. Since the interpretations were controversial, the feeding study should have been repeated, including more generations to address developmental questions as well, since - as mentioned above - organisms are most sensitive during their development and during substantial changes such as gestation and lactation periods. Due to these lacks in the presented scientific data of the single events, it is not regarded as apropriate to apply for approval of the multi-stacked event before clarifying the shortcomings of the single events.	
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	Concerning GM maize NK603 Feeding studies with Angus-continental cross steers were performed (Erickson et al., 2003). There was no difference in the dry matter intake (DMI), but a small difference in the average daily growth (ADG), resulting in a slightly lower DMI/ADG relation for the GM corn fed group. The longissimus muscle measured between the 12th and 13th ribs was slightly shorter in the GM group (85,8 vs 89,7 cm). These differences were not statistically significant (p = 0,08 and p = 0,09), but even small indications should be noted and compared with other GMP agronomic feeding studies, since in these relatively short and parameter-limited studies even hints of potential adverse effects are important. No other differences were found in this study. A 90 day feeding study with rats investigating the effects of GM corn NK603 in two concentrations,11% and 33%, as compared to the parental line, also 11% and 33%, and six commercial hybrids, 33% only (Dudek, 2001). Significant differences between the test and control groups were compared to the population of reference controls and if the significant difference was not corroborated by this final comparison it was not considered biologically meaningful. The total number of test animals was 400 (200 per sex) in 10 groups, resulting in 40 rats being fed the GM test hybrid at 33%, as is normal for rodent diets, 40 rats fed with 11% GM corn diet and 320 rats with	The single events (59122, 1507 and NK603) as well as the double stacked events 59122 x NK603 and 1507 x NK603 have been the subjects of earlier assessments and have received EFSA GMO Panel scientific opinions (EFSA, 2003a, 2003b, 2004a, 2005a, 2005b, 2006c, 2007b, 2008). Maize 59122 was authorised under Regulation (EC) No 1829/2003 with Commission Decision 2007/702/EC (EC, 2007). Maize 1507 was authorised under Directive 2001/18/EC by Commission Decision 2005/772/EC (EC, 2005b) for feed use, import and processing. The placing of 1507 maize on the market for food use received authorisation under Regulation 1829/2003 with Commission Decision 2006/197/EC (EC, 2006). Maize NK603 was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC (EC, 2004). The use of food and food ingredients from NK603 maize was authorised under the Regulation (EC) No 258/97 (EC, 1997) by Commission Decision 2005/448/EC (EC, 2005a). The Panel is not aware of any new information that would change its opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			conventional corn diets. It is not clear why two different doses were used. Two doses would be insufficient to investigate dose-related effects and so far other studies (e.g. oral toxicity studies) showed no indication of dose-related impacts at very high concentrations of the novel proteins. Furthermore a non-linear dose-response shape could be appropriate. The corn was analysed for pesticide residues, but the contents were below the assay detection limit, except for chlordane which was higher than the allowed MRL. It is not clear whether the diet was fed as powder or in the form of pellets? Heat treatment changes proteins Statistically significant differences between the test and control groups: • Body weight gain was generally higher in the test group. In the 2nd week body weight gain of the male and in the 4th and 9th week for the female rats was significantly higher in the 33% GM fed than in the 33% control group, but not significant to the reference groups. In the 4th week this significant difference between the males also concerned the reference groups. Works both ways: body weight gain for the 33% GM fed group was significantly higher as compared to reference groups, but not to the control group and is therefore not considered important. • Feed intake was generally higher in the test group, some differences were significant. • Elevated levels of MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) in the test group were not considered of biological significance since both values are calculated from other calculated data – hematocrit/red blood cells and hemaglobin concentration/red blood cells. The conclusion is, that the elevated levels were caused by a slightly lower red blood cell count in combination with a slightly higher hematocrit or haemoglobin concentration at that sampling point. • Higher levels of lymphocytes, platelets, hematocrit, and mean corpuscular concentration as well as lower levels of neutrophils and monocytes • The clinical chemical parameters	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			albumin, blood urea, creatinine, alkaline phosphatase, chloride, phosphorous and calcium were lower, potassium higher. • The organ weights showed higher liver and heart weights in males. Unfortunately kidney weights were not included.	
Austria	Ministry of Health, Family and Youth	D, 07.08 Toxicology	The notifier argues that Bacillus thuringiensis derived proteins (Cry34Ab1, Cry35Ab1, Cry1F) have a history of safe use. However since the introduced traits are not originating from a commonly food source a safe history of consumption may not be deduced. For safe history of use see comments to chapter 7 (Allergenicity). Concerning GM maize 59122 A 90 day rat (Crl:CD (SD)IGS BR) feeding study has been carried out. Diet analyses showed that the diets were equivalent and in the ranges known for maize and maize hybrids. In test group fungal evaluation exceeded limits of 500 CFU/g but was still within the accepted limits according to the guide lines of US FDA/USDA for animal diets. Cry34Ab1 and Cry35Ab1 were only found in the test diet and remained stable over 90 days. But this concentration stability is questionable, since only 70 % of Cry34Ab1 were detected by PCR analysis at the end of the trial. Mean body weight and body weight gain as well as food consumption and food efficiency were within normal ranges. No adverse clinical signs and differences in survival occurred between the groups. Ophthalmologic and neurobehavioral evaluation revealed no differences. When it comes to the clinical pathological evaluation significant differences were observed for some traits: According to the authors the increase of total protein was due to an increase of albumin. No consideration was given to the globulin fraction, that is also found in total protein. Immunoglobulins (Igs) are increased in an activated immune status such as inflammation, allergy or autoimmune disease. Next to the cellular immune screening an evaluation of the humoral immune system (Igs evaluation) could be of interest. Furthermore there were differences concerning	See section 5.1.4.1 The single event 59122 and newly expressed protein in this event have been already assessed by the GMO Panel. Maize 59122 was authorised under Regulation 1829/2003 with Commission Decision 2007/702/EC. The Panel is not aware of any new information that would change its opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			corpuscular elements of the blood. Platelets and retikulocytes as well as mean corpuscular haemoglobin concentration were decreased in the GM fed group as compared to control and reference groups. Unfortunately there was no analysis of the bone marrow samples, which might give further information about alterations in the hematopoetic fraction. Alterations of total protein and albumin levels as well as the decrease in total blood volume nevertheless could indicate kidney dysfunctions. But this assumption was not confirmed by gross pathology or by histopathology. To get a more pronounced evidence for the safety of the transgenic hybrid maize line, DAS 59122-7, it would be of great interest to repeat the trial and evaluate if the findings are reproducible. It is worth to be mentioned that a non-significant decrease in the white blood cell count was noted due to a small decrease in the lymphocyte count, which might indicate the onset of viral infection or stress. In addition findings of gross necropsy (page 125: males - stomach dilation in 10 out of 12; page 126: all males - chronic liver inflammation; page 131/156: males and females - hemorrhage thymus in 10 out of 12; page 150: females – stomach problems in 10 out of 12; page 167: females – different kidney problems 6 out of 12) could point to a less than optimal health state of all test rats.	
Austria	Ministry of Health, Family and Youth	D, 07.09 Allergenicity	• Digestion experiments in simulated gastric environments for introduced proteins are of limited significance with regard to the methods used [See for instance: Fu, T.J. (2002), Digestion stability as a criterion for protein allergencity assessment. Ann. NY Acad. Sci. 964:95-110]. Data according to guidance by FAO with reduced amounts of pepsin should be submitted additionally. • Heat stability data, e.g. for Cry34Ab1 and Cry35Ab1, are not conclusive because only loss of biological function and not degradation of proteins into non-allergenic breakdown products was assayed. • Bioinformatics analysis was not conducted	Single events and newly expressed protein in single events have been already assessed, including for allergenicity. The Panel is not aware of any new information that would change its opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			according to FAO/WHO criteria (window of 6 consecutive amino acids for homology comparisons). Instead other parameters (window of 8 consecutive amino acids for homology comparisons) were employed. This constitutes a less stringent approach. Analyses according to FAO/WHO guidance should also be submitted. More direct tests for allergenicity as recommended in Spök et al. (2005b)[Spök A., Gaugitsch H., Laffer S., Pauli G., Saito H., Sampson H., Sibanda E., Thomas W., van Hage M., Valenta R. (2005), Suggestions for the Assessment of the Allergenic Potential of Genetically Modified Organisms. Int. Arch. Allergy Immunol. 137: 167-180] are therefore considered necessary to be employed. But negative findings may not be an indicator of safety.	
Austria	Ministry of Health, Family and Youth	D, 07.09 Allergenicity	For the assessment of allergenic properties of the introduced proteins reference was made to the assessment of individual traits in parental GMO events. Furthermore mostly indirect evidence was used for the assessment. The indicators used were information on the allergenicity of the source material, homology-comparisons of novel proteins to known allergens, digestibility of test proteins in simulated gastric environments, the heat stability of test proteins, absence of glycosylation and a concluded low level of expression of proteins.	Single events and newly expressed protein in single events have been already assessed, including for allergenicity. The overall information provided by the applicant based on the weight of evidence approach that was applied allowed the panel to conclude that the allergenicity was unlikely. The Panel is not aware of any new information that would change its opinion
Austria	Ministry of Health, Family and Youth	D, 07.09 Allergenicity	The relevance of some of these parameters like expression levels in GM plant materials is questionable and not considered to be of indicative value with regard to safety: • Since no threshold levels for sensitisation to potential allergens can be established, the criterion that introduced proteins are expressed at lower levels than most common food allergens is not conclusive. Source materials are qualified as non-allergenic by the notifier. However this conclusion cannot be justified with a view to data suggesting an allergenic potential at least for Cry proteins [Bernstein L.I., Bernstein J.A., Miller M., Tierzieva S., Bernstein D.I. Lummus Z., Selgrade	See above

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			J.K., Doerfler D.L., Seligy V.L. (1999), Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides.Environ. Health Perspectives 107(7): 575-582; Doekes G., Larsen P., Sigsgaard T., Baelum J. (2004), IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: the BIOGART study. Am J Ind Med. 46(4):404-7.]. Bernstein et al. (1999) investigated immune responses occurring in farm workers exposed to Bt containing pesticides and found indications that exposure to Bt sprays may lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both. These finding could be corroborated by recent reports from India in connection with allergic reactions in workers handling Bt cotton (Source: Frontline 23(12), India, by Venkitesh Ramakrishnan http://www.hinduonnet.com/fline/stories/20060630004102200.htm date: 17-30 Jun 2006). Additionally reports have been published on a possible connection between the inhalation of Bt maize pollen and adverse effects in Philippine villages (Traavik & Smith, 2004 [Terje Traavik & Jeffrey Smith (2004): Bt-maize (corn) during pollination, may trigger disease in people living near the cornfield. http://www.mindfully.org/GE/2004/Bt-Corn-Human-Disease24feb04.htm]). Although these reports don't present scientific papers, in connection with the findings of Bernstein et al. it can at least be expected that detailed investigations on a scientific basis are conducted to follow up these indications. But so far these observations and results have not been included in any discussions or assumptions about the allergenicity of Bt toxins. Similarly the findings of Fares & El-Sayed (1998) and Vázquez-Padrón et al. (2000)[Fares and El-Sayed, 1998; "Fine structural changes in the Ileum of mice fed on Endotoxin-treated Potatoes and Transgenic Potatoes" Natural Toxins, Vol. 6, Issue 6, pages 219-233; Vázquez-Padrón RI, Gonzáles-Cabrera J, Garcia-Tovar C, Neri-	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			Bazan L, Lopéz-Revilla R, Hernández M, Moreno-Fierro L and de la Riva GA. CrylAc protoxin from Bacillus thringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun 2000, 271, 54-8.] are not discussed. In these studies structural changes in the mouse ileum were observed and it was found that Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. The general opinion published in GM risk assessment reports assumes that in the absence of receptors for the delta endotoxins of Bacillus thurengiensis on mammalian intestinal cells there are no risks to be expected. Furthermore it is stated, that no allergic reactions towards Bt toxins are known which is not true. It is generally observed that controversial results pointing to potential hazards are mostly ignored, which neither does help to obtain a comprehensivel and clear picture of GM risks nor to solve the controversial discussion about these novel products.	
Austria	Ministry of Health, Family and Youth	D, 07.10 Nutritional assessment of GM food/feed	GM Maize 59122x1507xNK603 is considered to be nutritionally equivalent to non GM-maize based on the comparison of certain constituents and based on results of broiler feeding study. A critical evaluation of the concept of Substantial Equivalence concluded that such assessments should rather be a starting point for risk assessments and not considered an endpoint itself [Umweltbundesamt (2002), Evaluating Substantial Equivalence - A Step towards Improving the Risk/Safety Evaluation of GMOs. Vienna, 19.-20. October 2001, Conference Papers, Band 032]. The overall conclusion that feed products from GM Maize 59122x1507xNK603 are substantially equivalent to and as safe as feed products from commercial maize cannot be deduced from the scientific data presented. We recommend submission of further evidence clarifying the causes for the identified significant differences to substantiate conclusions (see also comments with regard to toxicity).	See response above and section 5.1.7 of the opinion

Application EFSA-GMO-UK-2005-21 (Maize 59122x1507xNK603) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Austria	Ministry of Health, Family and Youth	D, 10 Potential changes in the interactions of the GM plant with the biotic…	For environmental risk assessment only unintended release of GM Maize 59122x1507xNK603, e.g. accidental spillage, is considered. It is not clear which specific routes of unintentional release are considered for the conclusions of the notifier. It is therefore not clear whether cultivation of maize seed contaminated with GM Maize 59122x1507xNK603 or the effects of transgenic materials still present in faeces of animals fed with GM Maize 59122x1507xNK603 products were considered. Regarding conclusions of the notifier concerning effects on human health and animal health (see comments to chapters Comp. Analysis, Agronomic parameters, Toxicology, Allergenicity, Nutritional eqivalence). The conclusions do not seem to be justified based on data submitted in the dossier. More data should be submitted concerning: • tests with appropriate controls in comparison with GM Maize 59122x1507xNK603, • further empirical evidence concerning the observed statistically significant differences for compositional analyses (comparative assessment) and nutritional equivalence of GM Maize 59122x1507xNK603, • direct evidence concerning potential toxicological and allergological effects of GM Maize 59122x1507xNK603. Otherwise the conclusions by the notifier need to be rejected.	The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x 1507 x NK603 and does not include cultivation. Considering the proposed uses of maize 59122 x1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment.
Austria	Ministry of Health, Family and Youth	D, 12.01 General	Case specific monitoring The applicant concludes that based on the submitted risk assessment no identified adverse effects to humans and animals are to be expected. Therefore case-specific monitoring is not deemed appropriate. However, based on the identified shortcomings of the respective assessment this conclusion needs to be better justified. General surveillance The General Surveillance plan is too general in nature. The assertion of the notifier that Chapter 2 of the monitoring plan can be regarded a “detailed description of the proposed methods for general surveillance” must be rejected. The plan should better specify the surveillance system involved, with regard to the potential risks that are	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion.

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			not addressed in full in the risk assessment by the notifier and the measures, which would enable the participating networks to report any specific observations on adverse effects. Specifically for the monitoring of animal health the notifier need to present more details with regard to institutions approached. No information is given how unanticipated adverse effects of spillage could possibly be covered by the proposed monitoring. The monitoring plan therefore has to be considered insufficient. Descriptions of procedures and institutions involved are missing, as well as specific criteria for observatory measures. No information is contained, what is regarded to be an adverse effect, or how effects should be evaluated. No outline is given, how such information is collected and presented, who is collecting this information, and what knowledge and expertise involved persons should have. In conclusion, it is not clear how unanticipated effects in the environment, human and animal health will be accounted for under the general surveillance plan proposed. The reporting period is proposed to be 3 years for the first report, and possibly for additional following reports. No criteria are specified for setting this frequency of reporting, which seems overly long compared with other monitoring plans and not in line with the requirement of Directive 2001/18/EC. In conclusion, the proposed monitoring plan for GM Maize 59122x1507xNK603 is insufficient and inadequate for the purpose of general surveillance. The submitted monitoring plan therefore should be rejected.	
Belgium	Belgian Biosafety Advisory Council	A. General information	Comment 1 The fact that: - on the hand 59122 x 1507 x NK603 was obtained by traditional breeding methods between progeny of two genetically modified maize lines, and that no new genetic modification has been introduced in 59122 x 1507 x NK603 maize - on the other hand: - NK603 maize was considered as safe as conventional maize and that it therefore could be placed on the market for food or feed or	This summary is in agreement with Panel's opinion.

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			processing without an adverse effect on human or animal health or on the environment (EFSA, 2003) - the submission of an application for authorisation of genetically modified 59122 maize and derived food and feed under Regulation (EC) N° 1829/2003, and the conclusion that 59122 maize is as safe as its non genetically modified counterparts with respect to potential effects on human and animal health or the environment (EFSA, 2007) - EFSA (2005) considers that 1507 maize will not have an adverse effect on human and animal health or the environment in the context of its proposed use. may be an advantage with regard to the evaluation of the application of 59122 x 1507 x NK603 maize. This dossier is characterized by a holistic, integrative approach.	
Belgium	Belgian Biosafety Advisory Council	A. General information	Comment 2 see comment 2 under A for application 2005/20 Comment 3 Even if the two parents of the hybrid GMO 59122xNK603 were safe this does not prove that the hybrid is safe as there could be interactions between the transgene proteins That's why toxicity analyses on the real hybrid GMO are necessary. As 59122xNK603 will enter in the food chain as normal maize it'll probably also enter in the diet of mothers and kids. Therefore toxicity studies are lacking on gravid animals to assess possible teratogenic effects as well as the effects on neonates. Maize is usually consumed all over the year and doesn't present a seasonal ingestion so that humans and animals will be exposed to 59122xNK603 for long periods of time even all life long. The duration of toxicity assays are therefore too limited and should be prolonged for more that 90 days to assess chronic effects.	The GMO Panel considered the fact that 59122x1507xNK603 maize combines two traits conferring tolerance to different herbicides targeting amino acid metabolism. On request by the Panel the applicant presented overview tables summarising levels of crude protein and individual amino acids in the stack, the single events and corresponding non-GM comparators. It was demonstrated that crude protein and amino acid levels in the stack fell well within the respective ranges observed for the single events and/or the non-GM controls. Amino acid levels in the stack calculated as percentage of total amino acids were not consistently different compared to the non-GM control. In general, the levels of those compounds which were different to the level in the corresponding control were within the literature ranges reported for commercial maize varieties. As the comparison of the level of the various key constituents in 59122x1507xNK603 maize and its non-GM control did not reveal any statistically significant difference for constituents for which a food safety concern could be foreseen, the GMO Panel accepted that none of the field trial sites was replicated the second year.

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Belgium	Belgian Biosafety Advisory Council	C. Information relating to the genetic modification	What exactly is the difference between the plant EPSPS and the EPSPS from Agrobacterium CP4 so that glyphosate, the active component in Roundup, does not block CP4-EPSPS but does so with the plant EPSPS ?	The information regarding this issue can be found in Padgette et al., (1995) Crop Sci 35:1451, among others.
Belgium	Belgian Biosafety Advisory Council	D, 01 Description of the trait(s) and characteristics which have been introduced…	Pioneer Hi-Bred International, Inc. conclude that "there were no statistically significant differences between 59122xNK603 and non-GM control maize with comparable genetic background that fell outside the normal ranges of variation for commercial maize".In the annex 5, statistical differences can be observed in some amino acids, minerals, vitamins, …	Although differences between 59122xNK603 maize and non-GM control maize were occasionally observed, the GMO Panel agrees on the fact that no differences fell outside the natural variability observed for commercial maize lines. Based on the results of comparative analysis it is concluded that 59122xNK603 maize is compositionally and agronomically equivalent to conventional comparators, except for the presence of Cry34Ab1, Cry35Ab1, CP4EPSPS and PAT proteins in 59122xNK603 maize. See opinion on AP 20
Belgium	Belgian Biosafety Advisory Council	D, 02 Information on the sequences actually inserted or deleted	Comment 1 Appropriate molecular approaches should be used to assess intactness of the stacked transgene events. Southern blot analysis has been performed but for the NK603 insert, the enzyme/probe combination only can detect internal fragments. Therefore, this analysis does not confirm the intactness of the borders of the insert in the stacked hybrid maize. Concerning the Southern blot analyses, the plasmid controls do not always behave as expected: the CP4EPSPS is much weaker than would be expected from a positive copy control, the cry34 control is even invisible in figure 11 (annex 2); in contrast the pat and cry1F controls are often much stronger. Do the applicants have an explanation for this? Comment 2 PartI / P15: I did not find back the details of the results that back up the statements made in the last paragraph on p15, concerning the detailed analysis of the DNA flanking regions at both the 5' and 3' borders of the 1507 insert. Therefore I could not fully assess the information on the sequences actually inserted (including flanking genomic regions) for the 1507 insert. However, the reader is referred to Annex 5 (the sequence itself), to the Annex 1b folder in which a summary of the characteristics of the 1507 maize is described by an	Additional information has been requested on the intactness of the NK603 insert in the hybrid. The intactness of the NK603 insert was demonstrated by Southern analysis of *Msc*I and *Sca*I-digested DNA. For the molecular analysis of the hybrid the direct comparison with the respective parental lines is more important than the plasmid control. The fact that the reaction of the plasmid controls differs in strength is not considered to be a safety issue. Events 1507 and NK603 were previously assessed for their safety. Information on the inserts and flanking sequences are provided in the original dossiers.

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			EFSA panel, and to previous authorizations to place the 1507 maize to the market. Therefore 1507 event is considered safe. The information on the inserted sequence + flanking maize genomic regions in NK603 was not assessed, because for this information, reference is made to an earlier notification.	
Belgium	Belgian Biosafety Advisory Council	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	Comment 1 Why is one of the three transgene inserts (1507) segregating and the others not in the seeds of the hybrid with stacked events? Are the non-segregating inserts homozygous in this line? Furthermore it is not really explained how this triple stacked 'hybrid' was obtained ('through breeding'). I would like the applicants to be a bit more clear on this point. The diagrams in dossiers EFSA/GMO/UK/2005/20 and 21 are exactly the same, while the hybrids differ. The diagrams representing how the hybrids are made (Annex 13) are speculative. Besides, according to these diagrams the transgenes end in different genetic backgrounds. As a consequence the "hybrids" that are used in animal trials are not the same as the "hybrids" that are used in agronomic performance+composition+expression trials. Although these confusing situations do not by definition provoke performance differences, it is a scientifically incorrect procedure. It is impossible to make a commercial hybrid if one works as indicated in the diagrams; as a consequence we expect the commercial hybrid to be different again from the tested material. So the commercialized product will not be genetically equal to the tested products. Again this is scientifically not correct. Comment 2 SNPs, sequencing and Microarray method exist to evaluate modification of gene expression. These new technologies which are much more accurate must be introduced in the panel of tests used to determine the eventual effects of a GMO in tissue.	Pedigree information regarding stacked events is not a requirement according to the Guidance Document on Stacked Events and not relevant for the genetic stability of the insert and phenotypic stability of the GM plant. All new technological advances which might add value to the risk assessment process and which are fully validated will continue to be considered by the GMO Panel
Belgium	Belgian Biosafety Advisory Council	D, 07.03 Selection of compounds for analysis	Comment 1 Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS proteins were bacterially produced (Annex 16). It has been mentioned that testing bacterial surrogate proteins should not substitute for testing	Since equivalence between plant and bacterially expressed proteins had been established by the applicant the GMO Panel accepts material from the both sources for use in the safety studies.

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			the plant-expressed proteins (Freese & Schubert, 2004). Freese, W., Schubert, D. 2004. Safety testing and regulation of genetically engineered foods. In Harding, S.E. (Ed.) Biotechnology and Genetic Engineering Reviews 21: 299-324. Comment 2 Statistically significant differences between 59122 x 1507 x NK603 maize and the non-GM control maize were observed for phosphorus, potassium, oleic- and linoleic acid, tryptophan, methionine and Vit E. However, on a per location basis, these differences were not consistently observed. All values in 59122 x 1507 x NK603 maize grain and non-GM control maize were within reported literature ranges.	
Belgium	Belgian Biosafety Advisory Council	D, 07.04 Agronomic traits	The 59122 x 1507 x NK603 maize was tested in the USA and Canada during the 2003 growing season; another genetically different version was tested in Chile during 2002-2003. The results obtained confirmed that there are no unexpected agronomic differences between the 59122 x 1507 x NK603 maize and non-GM-maize with comparable background. Results of 1 testing season are never conclusive since there is no opportunity to test potential year effets. And the material tested in Nord America was genetically not the same as the material tested in South America.	Given that the single events have been evaluated and found to be safe, the GMO Panel considers that one season of field trials is sufficient to demonstrate the compositional equivalence of the GM plant containing stacked events with its comparators (see references, EFSA 2007). The GMO Panel is not aware of agronomic trials performed with 59122x1507x NK603 maize in Chile.
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 1 - no homology with known toxins for Cry1F, Cry34 x Cry35 x PAT-protein expressed in 59122 x 1507 x NK603 maize. - no indication for any toxicity in vivo in acute toxicity tests with doses many times higher than normal uptake by man in the highest possible ("worst") scenario. - NK603 maize is resistant or tolerant to glyphosate, the active component in Roundup. The phosphonomethyl-glycine blocks the activity of 5-enolpyruvylshikimate-3-phosphate synthase or EPSPS, which is a key enzyme in the shikimic pathway leading to the formation of aromatic amino acids (tyrosine, phenylalamine and thryptophane) in plants, bacteria and fungi, but not in animals. Why then in some text books or dictionaries a low toxicity in animals is	With regard to the safety assessment of the single events and the likelihood for potential interaction of the transgenic proteins: see responses above. Concerning the relevance of the feeding study: see text in the opinion and response above. The safety of the newly expressed protein has been assessed previously in the opinions on the parental single events. The processing including temperature was not considered to have an impact on the safety of the newly expressed proteins. With regards the comment referring to the Cartagena Protocol, the Panel makes it clear that Cry proteins are INactivated at 90°C

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			mentioned ? Has the enzyme EPSPS other known functions ? Or is the term “low toxicity” misused ? Comment 2 The effect on the growth rate and feed intake of rats was tested. The number of animals used in the trial was sufficient for the female rats, but not for the male rats, due to a different variability within both sexes (Berndtson, W.E., J. Anim. Sci. 69, 67-76, 1991). Comment 3 The transgene proteins PAT, CP4 EPSPS, CrY1F and Cry34Ab1 + Cry35Ab1 were tested separately and not together; this does not give the opportunity to have data of possible interactions between these proteins. Only acute studies were done, some effects can only be seen after a long period of exposure so chronic studies are needed. Moreover these studies were done with the two parents of the hybrids and not with the hybrid under application. The data were not collected by independent labs!	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 4 Contents of CP4 EPSPS proteins presented in technical dossier (UK/2005/21; part I, pg. 20)are expressed as concentrations on fresh weight, while in annex 6 (table 54)data are expressed on dry weight. Where do the fresh weights come from ? Comment 5 This acute study is too short to observe long term effects. A chronic study should be conducted. Further study should be conducted to understand the effect of the GMO on abdominal fat and kidneys.	See section 3.1.4
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	References Bannon,G., Fu, T.J., Kimber, I., Hinton, D.M. 2003. Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124. Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami O.,, Shimada N.,, Guruge, K.S., Saito, M., Nakajima, Y. 2003. Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci. 81: 2546-2551. FAO/WHO, 2001. Evaluation of allergenicity of genetically modified foods: Report of a Joint FAO/WHO Expert Consultation on Allergenicity	Thank you for this summary of references.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			of Foods Derived from Biotechnology. FAO, Rome, 27pp. [http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf]. Herman, R.A., Storer, N.P., Gao, Y. 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154–1157. Spök, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, W., Valenta, R. 2005. Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int. Arch. Allergy Immunol. 137:167-180. Ladics et al Regul Toxicol Pharmacol 2006;44:136-43 Hoff et al. Mol Nutr Food Res 2007; 51:946-55 Pastorello et al. J Allergy Clin Immunol 2003; 112;775-83 Pasini et al. Allergy 2002; 57:98-106 Weichel et al. Allergy 2006;61:128-35	
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	Comment 1 FAO/WHO (2001) proposes pepsin degradation as a method for the evaluation of allergenicity of genetically modified foods. Furthermore, the similarity of amino acids with know allergens was studied as described by FAO/WHO (2001), where a cross-reactivity between the expressed protein and a known allergen has to be considered when there is: 1) more than 35 % identity in the amino acid sequence of the expressed protein, using a window of 80 amino acids and a suitable gap penalty, or 2) identity of 6 contiguous amino acids. However, there is no proof that a six or eight amino acid match is predictive in the bioinformatics section. A number of people now recommend not performing the 6-8 amino acid match. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used to test the digestion of Cry34Ab1 and Cry35Ab1 proteins (Annex 8i), PAT protein (Annex 9) and CP4 EPSPS protein (Part I of the dossier, p. 37). It has been shown that a rapid in vivo degradation of Cry proteins (Cry1Ab) does not always occur (Chowdhury et al., 2003). The fact that major allergens with high percent allergenicity were not necessarily more resistant to SGF or SIF digestion than allergens with	The Panel is aware of the publications quoted in the comment. Single events and newly expressed protein in single events have been already assessed, including for allergenicity using the strategy (i.e. weight of evidence approach) described in the EFSA guidance document. No new information would prompt the Panel to change its previous opinions. In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would in particular impact on the allergenicity. With regards the allergenicity of the whole plant, the panel is aware of the rare cases of allergy to maize, which however is not considered a common allergenic food. The Panel sees no reason to consider that the allergenicity of the GM maizes (e.g. the single events already assessed as well as the present stack event) would be changed because of the genetic modification

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			low percent allergenicity renders the use of SGF and SIF digestibility difficult as a tool to distinguish potential food allergens from non allergenic proteins (Fu et al., 2002). Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.	
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	Comment 2 Pioneer argues that the donor organisms have no history of causing allergy but as these organisms are soil bacteria it's obvious that these organism were not included in a normal human diet so that couldn't have provoked allergies. Moreover Pioneer claim no allergenicity for the new proteins because they don't share amino acids sequences with known allergens but again these proteins are new in human alimentation and so there is a need of specific scientific studies. Comment 3 As mentioned by the applicant, Cry34Ab1, Cry35Ab1, and PAT are not likely to be allergenic proteins. Cry1f, due to very low similarity with Der p 7, a mite allergen, has been further investigated by the applicant, but does not seem to have allergenic potential (Ladics et al., 2006). CP4 EPSPS has already been demonstrated to share some sequence similarity with Der f 2, a major allergen of the mite Dermatophagoides farinae. A recent report, however, concluded that there is no evidence of increased allergenic potential for CP4 EPSPS (Hoff et al. , 2007). As rightly mentioned by the applicant, food allergy to maize is rare. Some allergens have been determined (Pastorello et al., 2003; Pasini et al., 2002), and new allergens might be described in the near future (Weichel et al., 2006). Although the newly introduced proteins are not likely to be allergens, and although the parent plants do not seem to have increased allergenicity, their breading gives rise to what can be considered as a new plant, with potentially new molecular	See above

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			interactions. Theoretically, this might cause some modification in the expression levels of some maize proteins, including allergens. Therefore, it is the feeling of the reviewer that it might be relevant to analyze whether the allergenicity of the whole new plant is increased, compared to its traditional counterpart.	
Belgium	Belgian Biosafety Advisory Council	D, 07.10 Nutritional assessment of GM food/feed	I did not find any information dealing with the in vitro organic matter digestibility of 59122 x 1507 x NK603 maize. This is a rapid technique that can provide interesting information. Based on the chemical composition and the vitro organic matter digestibility, the metabolic and net energy can be estimated, yielding extra information for pigs and ruminants. In the poultry feeding study feed efficiency was not different, which may be an indication of a similar digestibility of GM and control maize. Annex 12 (p.22) mentioned the presence of CP4 EPSPS protein in 2 out of 6 control diet samples. So, the control diet were not really a negative control. On the other hand, the fact that these results did not show a detrimental effect on the chickens may provide some guarantee. This may be a reflection of a practical situation where novel proteins in the diet may not only come from 59122 x 1507 x NK603 maize, but also from GM soybean meal, wheat, ...	Concerning the relevance of the feeding study in the context of the present application: see text in the opinion and response above.
Belgium	Belgian Biosafety Advisory Council	D, 08 Post-market monitoring of GM food/feed	As no long term toxicity studies has been done, we can not exclude long term effect of OGM consumption. That's why it is required a follow-up of the GM food post-market.	The risk assessment concluded that no data have emerged to indicate that maize 59122 x 1507 x NK603 is any less safe than its non-GM comparator and parental GM lines maize lines. In addition, maize 59122 x 1507 x NK603 is, from a nutritional point of view, equivalent to conventional maize. Therefore, in line with the Guidance document (EFSA, 2006a), the GMO Panel is of the opinion that post-market monitoring of the food/feed derived from 59122 x 1507 x NK603 maize is not necessary.
Belgium	Belgian Biosafety Advisory	D, 12.01 General	We support the view of ACRE in its annual report of 2006 (ACRE, 2007; p.42) that provision of the detailed arrangements for general surveillance post-	This comment partially falls outside the remit of the GMO Panel. Decision-making for specific conditions for placing a GMO on the market is left to the risk-

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	Council		market monitoring plans should be made a condition of any consent.	managers (e.g. European Commission and Member States). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
Denmark	Danish Forest and Nature Agency	D, 11 Potential interactions with the abiotic environment	Comment on point D, 11.5 in the application : The applicant proposes to submit a first report on unanticipated adverse effects of the import and processing of 59122x1507xNK603 maize after 3 years following its authorisation. However, the reporting of such effects should be done on a yearly basis. Comments on point D.11.3 and D.11.4 in the application: It is suggested that monitoring of unanticipated adverse effects of the 59122x1507xNK603-maize is done each year and that monitoring activities and results from single years are included in the report which is compiled for the competent authorities every third year. Possible adventitious presence of 59122x1507xNK603-maize in other maize seed lots should be included in the monitoring plan due to potential co-existence problems.	See section 6.1.3 of the scientific opinion
Finland	Board for Gene Technology	General comments	We want to emphasize the need of high quality of general surveillance plan when adopting the product in a specific country.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the

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				applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
France	MINEFE - DGCCRF	D, 02 Information on the sequences actually inserted or deleted	(D) Informations relatives à la plante génétiquement modifiée (2) Les analyses de type Southern, utilisant une large gamme d'enzymes de restriction et de sondes spécifiques des inserts 59122, 1507 et NK 603, montrent que les inserts présents chez l'hybride correspondent bien aux inserts hérités de chacun des parents, que la structure moléculaire des inserts tels que décrits chez les parents est préservée chez l'hybride obtenu par croisement conventionnel et que les inserts sont situés dans le génome nucléaire de l'hybride. Cependant qu'aucune information n'est donnée sur le mode de constitution de l'hybride porteur des trois événements de transformation. Automatic translation: (D) Information relating to the genetically modified plant (2) the analyses of the Southern type, using a broad range of enzymes of restriction and specific probes of inserts 59122, 1507 and NK 603, show that the inserts present at the hybrid correspond well to the inserts inherited each parent, that the molecular structure of the inserts as described in the parents is preserved at the hybrid obtained by conventional crossing and that the inserts are located in the nuclear genome of the hybrid. However that no information is given on the mode of constitution of the hybrid carrying the three events of transformation.	The hybrid 59122x1507xNK603 is constructed by conventional breeding.
France	MINEFE - DGCCRF	D, 07.08 Toxicology	(7.8.4) Etude de toxicité subchronique Maïs 59122 x 1507 x NK 603 Aucune étude de toxicité	Given the results of the compositional analysis, the functional properties of the newly expressed proteins,

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			subchronique n'a été réalisée chez le rat avec le maïs hybride 59122 x 1507 x NK 603 mais que, compte tenu du fait que : - des études de toxicité subchronique de 90 jours ont été réalisées avec les maïs parentaux 59122, 1507 et NK 603 et qu'aucun effet délétère n'a été observé chez l'animal pour ces maïs, - aucun effet toxique ou délétère chez l'animal de laboratoire n'a été mis en évidence pour les 5 protéines d'intérêt, - les niveaux d'expression des protéines d'intérêt, compte tenu des écart-types observés, n'étant pas modifiés chez l'hybride comparés aux niveaux mesurés chez les parents, un tel élément est en faveur d'une absence d'interaction entre les événements de transformation, - une étude d'alimentarité a été réalisée chez le poulet qui permet de conclure à l'équivalence nutritionnelle du maïs hybride avec son témoin, il est possible de considérer que ces éléments, notamment les résultats des trois essais de toxicité subchronique sur chacun des maïs parents, sont suffisants pour démontrer l'innocuité des produits de l'hybride 59122 x 1507 x NK 603. L'Agence française de sécurité sanitaire des aliments considère qu'au regard notamment des données sur l'analyse des résultats de composition chimique, les données de toxicité chez les parents et de l'étude d'alimentarité chez l'animal cible, les produits dérivés des variétés de maïs portant dans le même génome les événements de transformation 59122, 1507 et NK 603 présentent le même niveau de sécurité sanitaire que le maïs conventionnel et ses produits dérivés. Il convient cependant de noter qu'aucune information n'est donnée sur le mode de constitution de l'hybride porteur des deux événements de transformation. Cette information, même si elle n'affecte pas l'évaluation des risques de cet organisme génétiquement modifié, devrait être fournie dans le dossier. En effet, dans ce type de dossier où les empilements de gènes sont plus nombreux, une telle information devient nécessaire pour rendre transparente au plan de la génétique formelle les	and the additional information provided by the Applicant, the Panel concluded that 59122 x 1507 x NK 603 maize is compositionally and agronomically equivalent to conventional maize lines and that interaction between the newly expressed proteins that could cause changes in the composition or food/feed safety of the 59122 x 1507 x NK 603 maize are unlikely. Therefore additional toxicity studies, e.g. 90 day subchronic toxicity study on rodents with the 59122 x 1507 x NK 603 maize, are not considered necessary in accordance with the EFSA Guidance document.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			constitutions génétiques et mieux comprendre la pertinence des témoins. Automatic translation: 7.8.4) Study of subchronic toxicity Maïs 59122 X 1507 X NK 603 No study of subchronic toxicity was carried out in the rat with hybrid corn 59122 X 1507 X NK 603 but that, taking into account the fact that: - studies of 90 days subchronic toxicity were carried out with the parental corn 59122, 1507 and NK 603 and that no noxious effect was observed in the animal for these corn, - no toxic or noxious effect in the animal of laboratory was highlighted for 5 proteins of interest, - the levels of form of proteins of interest, taking into account the standard deviations observed, being modified at the hybrid not compared at the levels measured in the parents, such an element is in favour of an absence of interaction between the events of transformation, - a study of alimentarity was carried out in chicken witness, it is possible to consider that these elements, in particular the results of the three tests of subchronic toxicity on each corn parents, are sufficient to show the harmlessness of the products of the hybrid 59122 X 1507 X NK 603. The French Agency of medical safety of food considers that at the glance in particular data on the analysis of the results of chemical composition, the data of toxicity in the parents and the study of alimentarity in the target animal, the products derived from the varieties of bearing corn in the same genome the events of transformation 59122, 1507 and NK 603 have the same level of medical safety as conventional corn and its derived products. It is however advisable to note that no information is given on the mode of constitution of the hybrid carrying the two events of transformation. This information, even if it does not affect the evaluation of the risks of this genetically modified organization, should be provided in the file. Indeed, in this type of file where gene stackings are more numerous, such an information becomes necessary to	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			be transparent in the plan of the genetics use to form the genetic constitutions and to better include/understand the relevance of the witnesses.	
Germany	Federal Agency for Nature Conservation (BfN)	General comments	Information (data and data analyses) provided on expression of the inserts, agronomic traits and composition is insufficient, and conclusions of substantial equivalence of 59122x1507xNK603 maize and commercial maize based on this information are premature. Although application EFSA/GMO/UK/2005/21 does not include the cultivation of 59122x1507xNK603 maize in the European Union, possible ecological consequences arising from accidental spillage or other forms of introduction of the transgene products in the environment should be considered more thoroughly. The applicant's proposal for an environmental monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC).	The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x 1507 x NK603 and does not include cultivation. Considering the proposed uses of maize 59122 x1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment. The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
Germany	Federal Agency for Nature Conservation (BfN)	D, 03 Information on the expression of the insert	According to the EFSA Guidance Document for the risk assessment of stacked transformation events (EFSA 2007), expression, among others, should be a focus of risk assessment to address interactions between the stacked events. Therefore, with regard to a final assessment of the expression of the inserts in 59122x1507xNK603 maize, a more robust and reliable data basis is required, including a higher	According to the Guidance of stacked events potential differences in the expression levels between the stacked line and the parental lines should be considered in ' *at least one year of field trial data is required, with trials ….in geographical localities representative of the climatic conditions under which such crops will be cultivated'.* Since no significant differences in expression levels are

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			number of replications per site and sufficient statistics. Since protein expression in plants can be affected by climatic conditions, soil fertility, agricultural practice or unknown gene-environment interactions, data from a single season (as provided by the applicant in Annex 6) give only a rough estimate of expression levels. A more robust and reliable data basis should, therefore, include data from at least three field seasons at the same location (with six locations representing different environmental conditions) to integrate possible differences in expression values triggered by differences in ecological conditions. EFSA [European Food Safety Authority] (2007): Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5.	demonstrated between the stacked line and the single events, one year of field trials and the choice for the main growing regions is considered appropriate by the EFSA panel.
Germany	Federal Agency for Nature Conservation (BfN)	D, 04 Information on how the GM plant differs from the recipient plant in: …	Although the agronomic characteristics addressed in Annex 6 of the dossier do not indicate a potential for differences in reproduction, dissemination, and survivability of 59122x1507xNK603 maize, the selected parameters themselves cannot sufficiently indicate such changes. Data presented on disease incidence and insect damage are of limited value because a range of pesticides were applied. The data set is based on a field design which is – because of the small plot size – not comparable to common agricultural practice. With regard to a final assessment, further information on reproduction, dissemination, and survivability is required, because the information provided (data from one season and six individual sites; Annex 6) is not considered sufficient to support the conclusion of substantial equivalence of 59122x1507xNK603 maize and commercial maize. The applicant should be asked to provide a robust and reliable data basis for reproduction, dissemination, and survivability to assess potential interactions between the events. Field studies with ecology-based parameters such as	The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x 1507 x NK603 and does not include cultivation. Considering the proposed uses of maize 59122 x1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			frost tolerance, seed dormancy, or competitiveness of 59122x1507xNK603 maize tested under field conditions should be included in the application. Relevant data should be collected to account for a minimum of three growing seasons and six locations representing different environmental conditions. The environmental conditions should be documented and provided with the application to assess their possible effects on the considered parameters. A summarising statistical analysis should address the between-site variation of the data. EFSA [European Food Safety Authority] (2007): Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.01 Comparative assessment	With regard to a final assessment, further information is required, because the information provided (data form one season and six individual sites; Annex 6) is not considered sufficient to support the conclusion of substantial equivalence of 59122x1507xNK603 maize and commercial maize. The applicant should be asked to provide a robust and reliable data basis for composition to assess potential interactions between the parental events. Plant material should be sampled during a minimum of three growing seasons and at six locations representing different environmental conditions. The environmental conditions should be documented and provided with the application. A summarising statistical analysis should address the between-site variation of all parameters. According to the EFSA Guidance Document for the risk assessment of GM plants (EFSA 2004), it is advisable that experiments with herbicide tolerant crops include GM plants that were not treated with herbicides. Therefore, the applicant is asked to provide composition data from 59122x1507xNK603 maize treated neither with glyphosate nor glufosinate herbicides. According to the EFSA Guidance Document for the risk assessment	The GMO Panel confirms that comparative analyses performed with 59122x1507xNK603 maize were conducted in agreement with the pertaining EFSA Guidance documents. Concerning the number of growing seasons, the use of single events as comparators, the inclusion of untreated GM plants in the field trials and the questions on potential interaction between newly expressed proteins: see responses above.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			of stacked transformation events (EFSA 2007), appropriate comparators for the GM plant containing stacked events should include parental GM lines. The applicant is asked to include the parental GM lines 59122 maize 1507 maize and NK603 maize in the study design at the same study sites. EFSA [European Food Safety Authority] (2004): Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-94. [Adopted on 24 September 2004; updated on 7 December 2005; final, edited version of 28 April 2006]. EFSA [European Food Safety Authority] (2007): Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.08 Toxicology	With regard to the applicant's references to the history of safe use of Bt toxins it should be considered that Bt strains producing the binary proteins Cry34Ab1 and Cry35Ab1 were never used in commercial microbial insecticides and only recently discovered (Moellenbeck et al. 2001). Due to the synergistic mode of action of the binary toxins depending on the ratio with Cry34Ab1 being the dominating factor (Herman et al. 2002), conclusions based on supposed but not proven analogies between other Cry toxins and binary toxins should take this into consideration. According to the EFSA Guidance Document for the risk assessment of GM plants containing stacked transformation events (EFSA 2007), toxicology, allergenicity and nutritional assessments as part of the food and feed assessment "clearly require a case-by-case approach" to consider potential effects that may arise from additive, synergistic or antagonistic effects of the gene products or their metabolites. This is consistent with potential interactions between the events being, among others, a main focus of the risk assessment of	With regard to the safety assessment of the single events and of the proteins newly expressed in the single events, the absence of necessity for additional toxicity studies, and the relevance of the feeding study provided by the applicant: see responses above.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			stacked transformation events. The applicant provided a poultry feeding study for the toxicology assessments. Although the poultry feeding study do not indicate a potential for toxicological effects, a complete assessment is not possible because of a lack of the individual data of Mortality and body weight. Further tests of haematology, clinical biochemistry and pathology are missing in the poultry feeding study. Hence, an because indications for possible adverse effects of 59122 and 1507 maize on mammals (statistical significant changes in haematology / serum counts of eosinophil leukocytes in female rats) were observed in earlier studies (Annex 9f of the dossier; MacKenzie 2003 in application EFSA/GMO/NL/2004/02), the applicant is asked to provide a second feeding study with the whole food and feed, i.e. 59122x1507xNK603 maize compared with maize with a comparable genetic background, on a case-by-case basis. We suggest conducting at least a 90-day feeding study with rodents addressing haematology, clinical biochemistry and pathology. EFSA [European Food Safety Authority] (2007): Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5. Herman RA, Scherer PN, Young DL et al. (2002): Binary insecticidal crystal protein from Bacillus thuringiensis, strain PS149B1: effects of individual protein components and mixtures in laboratory bioassays. J. Econ. Entomol. 95: 635-639. MacKenzie SA (2003): Thirteen-week feeding study with transgenic maize grain (TC1507) in rats. Moellenbeck DJ, Peters ML, Bing JW et al. (2001): Insecticidal proteins from Bacillus thuringiensis protect corn from corn rootworms. Nature Biotechnology 19: 668-672.	
Germany	Federal Agency for Nature	D, 10 Potential changes in the interactions of the	Water and soil organisms may be exposed to 59122x1507xNK603 maize via the release of organic waste material, litter or sewage to the environment,	The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x 1507 x NK603 and does

Country	Organisation	Reference	Comment	EFSA GMO Panel response
	Conservation (BfN)	GM plant with the biotic…	which occurs during processing or through spillage. No data are provided by the applicant about the concentration of the proteins Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS in organic waste material, litter or sewage. The possibility of an accumulation of the mentioned substances in the environment and of subsequent effects on water and soil organisms is not assessed. Therefore, the applicant is requested to provide data on this issue and to submit a risk assessment concerning the possible exposure of water and soil organisms to the mentioned substances.	not include cultivation. Considering the proposed uses of maize 59122 x1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	As stated by the applicant, the scope of the application of 59122x1507xNK603 maize is for import and processing and all uses for food and feed. The applicant's proposal for an environmental monitoring plan does not fully meet the requirements according to Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC. Therefore, a plan suitable to meet the objectives is requested. Both parts of the monitoring plan, the case-specific monitoring and the general surveillance, have to meet the following requirements: • Provision of a fully specified list of monitoring parameters: The applicant is requested to present for each parameter a detailed statement of the parameter definition, the observation methods (collection and analysis of samples with references), the frequencies of observations (time and number of visits to collect data) and the monitoring locations including number and size. Furthermore, an operating schedule giving full details of points in time is requested. • Determination of the baseline status of the receiving environment with respect to the monitoring parameters. • Elaboration of a sampling concept: Particularly, it must be explained how the necessary representativeness of the collected data in space and time is ascertained. The applicant is requested to indicate how the monitoring plan is adapted to different local conditions where appropriate. • Characterisation of reference areas. •	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			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. The monitoring should be run in regions, where 59122x1507xNK603 maize will be transported, processed or used. 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 of certain parameters beyond the period of the consent.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.02 Case-specific GM plant monitoring	We do not share the applicant's opinion, that a case-specific monitoring is not necessary. During transport, storage, packaging or processing incidental spillage of 59122x1507xNK603 maize can occur. Furthermore the exposure of 59122x1507xNK603 maize and the corresponding binary toxins to the environment during or after the production process and during animal consumption is given. Therefore, case specific monitoring has to focus on pathways, how the 59122x1507xNK603 maize can get into the environment. Related to the currently available data, the monitoring plan has to comprise the following elements: • exposure of maize kernels in the environment e.g. via spillage during transport, storage, packaging, processing and use, • spread, persistence and accumulation of 59122x1507xNK603 maize and the corresponding Bt-toxins if spillage or loss during transport, storage, packaging, processing and use occurs, • exposure of Bt-toxins in the environment, e.g. via sewage water, waste or by-products which occur during processing. If spread, persistence and accumulation of 59122x1507xNK603 maize and the corresponding Bt-proteins in the receiving environment or the exposure of the corresponding Bt-toxins in the environment, e.g. via sewage water, waste, by-products occur, further observations of possible impacts on organisms, food	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			chains and habitats are required. The monitoring plan appoints management systems, which should restrict further environment exposure of viable 59122x1507xNK603 maize in case of spillage. The applicant is requested to state in detail, how spillage and exposure of Bt-toxins in the environment will be detected and furthermore, in which way these management systems will be established and will work to restrict further environmental exposure in case of loss or spillage of viable grains of 59122x1507xNK603 maize.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	According to Directive 2001/18/EC general surveillance is a compulsory part of the monitoring. The objective of general surveillance is to monitor potential cumulative long-term impacts on human health and the environment and to identify the occurrence of adverse effects of the GMO on human health and the environment which were not anticipated in the E.R.A. The general surveillance plan has to focus on possible pathways how 59122x1507xNK603 maize can get into the broader environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal of the GMO. The applicant is requested to provide an appropriate monitoring plan to observe the spread, persistence and accumulation of the proteins Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS in organisms and environmental media (soil, air, water).	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.06 Reporting the results of monitoring	The monitoring results have to be reported on an annual basis. The applicant is requested to state, how the results will be published.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information

Country	Organisation	Reference	Comment	EFSA GMO Panel response
				provided. See section 6.1.3 of the scientific opinion. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan (on an annual basis).
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	General comments	The scope of this application covers import and processing of maize Event 59122x1507xNK603 including all feed and food products containing, consisting of, or produced from the genetically modified maize Event 59122x1507xNK603. Cultivation is not covered by this application. The German CA is of the opinion that further information is required to conclude the risk assessment of dossier EFSA/GMO/UK/2005/21.	
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	A, 07 Where appropriate, the conditions for placing on the market the food(s) or…	Appropriate measures have to be taken during transport, storage and processing to avoid unintended release into the environment. Labelling should include information indicating that 59122x1507xNK603 maize cannot be controlled by glyphosate or glufosinate-ammonium containing products.	The application EFSA-GMO-UK-2005-21 concerns food and feed uses, import and processing, but excluding cultivation in the EU. There are no indications of increased likelihood of establishment or survival of feral maize plants in case of accidental release into the environment of 59122 x 1507 x NK603 seeds during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of sporadic feral plants and the low levels of exposure through other routes indicate that the risk to target and non-target organisms is negligible. Labeling is not in the remit of GMO Panel.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 03 Information on the expression of the insert	Data on the expression of the inserts of the stacked events were obtained from field trials at six locations in the US and Canada in 2003. No appropriate comparator (parental lines) was grown in this field trial. Instead, obtained data for the stacked events were compared with data obtained from parental lines grown in field trials in the US, Canada, Chile and Europe at different locations and different growing seasons. This is not in line with the EFSA guidance document on stacked events. The dossier discusses expression analysis of the transgenes in grain only.	The applicant provided new data from European field trials with 59122x1507xNK603 in 2005, on five locations in Spain, Bulgaria and Hungary. Results on expression levels of the three Cry proteins, the PAT and the CP4 EPSPS protein demonstrated levels in the stacked line to be in the same range as in the parental lines. No effect was apparent for herbicide application. The GMO Panel considers these data on expression of the inserts sufficient for the safety assessment.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			Data on forage are available (annex 6 of the dossier), but not compared to appropriate comparators. EFSA (2007) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA Journal 512: 1-5.	The GMO Panel considers the information provided to be sufficient on the basis that the scope of the application covers only food, feed, import and processing
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.03 Selection of compounds for analysis	The comparative assessment in the dossier includes compositional analysis of grain only. Data on forage (as given in annex 6) is not discussed in the dossier. Furthermore, the cited OECD consensus document on compositional consideration for new varieties of maize (OECD, 2002) specifies more maize matrices in which nutritional and compositional parameters should be analyzed for human food use. In this regard, suggested nutritional and compositional parameters to be analysed in the following maize matrices for human food use are: oil („_ fatty acids), starch („_ proximate analysis), grits/meal/flour („_ proximate analysis, amino acids, fatty acids), and kernels („_ proximate analysis, minerals, vitamins, amino acids, fatty acids, phytic acid, raffinose, furfural, ferulic acid, p-coumaric acid). Hence, in this context the applicant should be asked to explain the choice of the investigated maize tissues and state a reason for the sufficiency of the presented results. OECD. (2002) Consensus document on compositional considerations for new varieties of maize (Zea Mays): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Organization of European Cooperation and Development, Series on the Safety of Novel Foods and Feeds, OECD ENV/JM/MONO (2002)25. The EFSA guidance document advises in the case of herbicide tolerant plants to include both, blocks of genetically modified plants exposed to the intended herbicide and blocks not exposed to the herbicide. The latter is missing in the field design of the 2003 field trials of the stacked events. At least, a statement by the notifier is requested of why non-sprayed plants were not	The GMO Panel considered the compositional data for forage provided in Annex 5 of the Technical Dossier. The Panel confirms the approach taken by the applicant to analyse raw commodities being representative for the various food/feed constituents produced from maize. Since grain and forage produced from 59122x1507xNK603 maize were shown to be compositionally equivalent to conventional maize lines, no further comparative compositional analyses are considered necessary. With regard to the inclusion of untreated GM samples: see above

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			included into the experiments. EFSA (2004) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-94.	
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.10 Nutritional assessment of GM food/feed	With regard to the poultry feeding study, the German CA is of the opinion that additional information is required: a) It is not clear from annex 12 how body weight of the animals was determined, clarification is required. b) Data on the yield of various parts of the carcass in annex 12 is presented graphically only, no raw data on the individual weights are available. The graphical presentation on the mortality rate is not clear at all, clarification is required.	The studies were carried out under standards GLP procedures and quality assurance. The Panel is of the opinion that the results presented by the applicant, including on mortality rate are sufficient and do not raise particular concerns.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 12.03 General Surveillance of the impact of the GM plant	The German CA is of the opinion that the general surveillance plan suggested in the notification is not sufficient. As part of the "active surveillance", it is planned to inform traders and processors as well as to gather information from different communication networks. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried and gathered. The use of questionnaires could be an appropriate measure to survey this information. 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. Furthermore, the applicant should specify monitoring activities in the field of human and animal health. Therefore, it should be described in more detail how animal and human health surveillance is integrated in the monitoring plan. A report on GS activities only every third year is insufficient. It is suggested to report on an annual basis about the conducted monitoring measures and every third year to provide an extended report with an overall analysis of the results from the last years.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			However, the monitoring reports should not only consist of general information from participating networks, but should also be analysed by the consent holder in more detail. In particular, indirect, long-term or cumulative effects could be detected after consideration of data from different networks and overall analysis over several years. Possibly single participating networks will not be able to take this aspect into consideration.	
Greece	Hellenic Food Authority (EFET)	D, 02 Information on the sequences actually inserted or deleted	Since the probes used in southern blots confirming the copy number, the structure and the organization of the inserts in 59122 X 1507 X NK603 are not adequately designed (they do not include flanking regions as proposed by EFSA guidance), additional molecular analysis data should be provided in order to further complete the molecular characterization. PCR analysis, southern blots using properly designed probes or sequence analysis of the insert and its flanking regions should be performed in order to confirm the organization of the insert into the hybrid genome.	Additional info has been requested on the intactness of the inserts and the flanks. Molecular equivalence of the 59122, 1507 and NK603 insert in the hybrid line was determined by Southern analysis, using *Sac*I, *Hind*III and *EcoRV* digested genomic DNA and probes of the *pat*, *Cry1F*, *Cry34Ab1*, *Cry35Ab1* and *cp4 epsps* genes. From the hybridisation patters of 59122x1507xNK603 and all parental lines it was concluded that the organisation of sequences in the insert are unchanged. Also the intactness of the 1507 insert and of the 3' side of the 59122 was confirmed. Additional information has been supplied on the intactness of the NK603 insert and the 5' of the 59122 insert in the hybrid line. The intactness of the NK603 insert was demonstrated by Southern analysis of *Msc*I and *Sca*I-digested DNA. The intactness 59122 insert in the hybrid line was confirmed by results obtained by event-specific real time tactness of the inserts and on flanking sequences
Greece	Hellenic Food Authority (EFET)	D, 03 Information on the expression of the insert	Expression level of PAT protein in the hybrid is compared separately with expression level of PAT protein in 59122 maize and with expression level of PAT protein in 1507 maize. It is found to be - in both cases - comparable to the level found in the separates single events. This comparison does not seem to be reasonable as the hybrid has two copies of the pat gene. How EFSA GMO panel comments this fact?	Considering that the potential toxicity of PAT has been extensively analysed, the variation in expression levels reported in the dossiers is not considered as a safety issue. In addition, expression levels are not always proportional to gene copy number.
Greece	Hellenic Food	D, 07.02 Field trials | D, 07.04	For the comparative assessment (agronomic, compositional and nutritional studies) as comparators	See sections 4.1.2 and 4.1.3

Country	Organisation	Reference	Comment	EFSA GMO Panel response
	Authority (EFET)	Agronomic traits | D, 07.10 Nutritional assessment of GM food/feed	were used only the non-GM nearly isogenic lines and commercial hybrids (Annex 6 and Annex 12) but not the GM parental lines as it is clearly indicated in EFSA's Guidance Document for stacked genes (The EFSA Journal, 2007, 512:1-5). There should be provided to EFSA the comparative assessment with the use of the GM parental lines as comparators.	
Greece	Hellenic Food Authority (EFET)	D, 07.08 Toxicology	Each of the introduced traits from the parental lines are inherited in 59122 X 1507 × NK603 maize, which results in the expression of the Cry34Ab1, Cry35Ab1, Cry1F, PAT and CP4 EPSPS proteins in the same plant. There should be conducted a toxicity study (a 90-day rat feeding study) with the whole of the 5 introduced proteins in the hybrid and not with each one transformation event (GM parents) separately.	The safety of the newly expressed proteins was assessed by the GMO Panel within the applications for the single events. In addition the overall information provided by the Applicant does not indicate possible interactions between those newly expressed proteins that would in particular impact on the food/feed safety. Since 59122 X 1507 × NK603 maize is agronomically and compositionally equivalent to its non-GM control, no additional toxicological study, e.g. 90-day subchronic toxicity study on rats is required to conclude on the safety of 59122 X 1507 × NK603 maize.
Italy	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	D, 08 Post-market monitoring of GM food/feed	To this Competent Authority a more detailed and comprehensive General Surveillance plan shall be described by the notifier. Referring to page113 paragraph 1:the management systems exemplified by the notifier in case of substantial unintended release of viable GMO object of this application seems to be not fully appropriate to this C.A.. The notifier should proposed a management procedure specific for GMO unintended releases. Furthermore referring to page 110 paragraph n.3 (effects on biogeochemical cycles) of the application, it should be clearified that " the expression of the Cry34 Ab1, Cry35Ab1,Cry1F , Pat and CP4EPSPS protein in 59122x1507xNK603 maize will not cause any possible immediate and/or delayed effects on biogeochemical cycles" since the scope of the application does not include cultivation.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
Malta	Malta Environment and	General comments	Malta does not have any comments on this application other than pointing out the difficulty to enforce such applications once approved.	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
	Planning Authority			
Norway	Norwegian Directorate for Nature Management	A. General information	According to the Norwegian Gene Technology Act possible contributions to a sustainable development and possible benefits to the society and ethical considerations through the use of a GMO, shall be taken into consideration when evaluating a GMO notification in Norway. In the case of notification EFSA/GMO/UK/2005/21 we request the Notifier to provide more information on these issues. Amongst others the Notifier should provide information on any changes in agricultural practices in the exporting countries that may have an impact on the environment and/or health. This would include effects (positive and negative) linked to the herbicide and pesticide regimes used when cultivating the 59122x1507xNK603 maize compared to non-glyphosate tolerant and insect resistant maize (e.g. what documentation exists on changes in farmers' exposure to agrochemicals, what information exists that documents effects on non-target species, particularly species of Coleoptera and Lepidoptera, arising from the changes in agricultural practices)	Outside Panel's remit. Human and animal health issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the GMO Panel.
Norway	Norwegian Directorate for Nature Management	D, 10.05 Interactions of the GM plant with non-target organisms	Does the Notifier have results from, or information of, field trials with the hybrid maize 59122x1507x NK603 where the aim of the trials has been to assess effects on non-target organisms (in particular invertebrates of Lepidoptera and Coleoptera)? For the parental line 59122 information in annex III of notification EFSA/GMO/NL/2005/23 is provided where a combined effect of the two CRY-proteins on the target organisms is shown. Has the notifier performed bioassays with the appropriate indicator species to check for possible combined effects of the three CRY-proteins in 59122x1507xNK603 on non-target organisms? Even though notification EFSA/GMO/UK/2005/21 does not include cultivation, accidental release (e.g. spillage, co-mixture with seed) may result in release of the hybrid into the environment; in which case information regarding	The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 59122 x 1507 x NK603 and does not include cultivation. Considering the proposed uses of maize 59122 x1507 x NK603, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			effects on non-target organisms, both through field trials and feeding studies is important in order to assess the impact on the environment.	
Norway	Norwegian Directorate for Nature Management	D, 07.01 Comparative assessment	The applicant states that the non-GM comparator has comparable genetics to 59122x1507xNK603. There is no outline about the process of rearing this non-GM comparator. Such an outline should be presented.	Additional information concerning the breeding scheme for the comparators used in the field trials.
Norway	Norwegian Directorate for Nature Management	D, 07.02 Field trials	Comparison between 59122x1507xNK603 and non-GM comparator should cover more than one growing season. Comparison between 59122x1507xNK603 and non-GM should also cover locations representative of various European environments where maize can be grown.	See responses above
Norway	Norwegian Directorate for Nature Management	D, 07.09 Allergenicity	Scientific studies, also very recent ones, have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant, which is an enhancer of immune responses. The GMO Panel of the Norwegian Scientific Committee for Food Safety find it difficult, based on the available data, to assess whether kernels from maize 59122x1507xNK603 may cause more allergenic reactions than food and feed from unmodified kernels. As the different Cry proteins are closely related, and in view of the experimental studies in mice, the GMO Panel finds that the likelihood of an increase in allergenic activity due to Cry34Ab1, Cry35Ab1 and Cry1F proteins in food and feed from maize 59122x1507xNK603, cannot be excluded. Thus, the Panel's view is that as the adjuvant effect of Cry34Ab1, Cry35Ab1 and Cry1F with reasonable certainty cannot be excluded, the applicant in relation to a possible adjuvant effect of Cry34Ab1, Cry35Ab1 and Cry1F must comment upon the mice studies showing humoral antibody response of Cry1A proteins. Further, although the Cry34Ab1, Cry35Ab1 and Cry1F proteins is rapidly degraded in gastric fluid after oral uptake, there is also the	Single events and newly expressed protein in single events, particularly Cry proteins) have been already assessed, including for allergenicity. The Panel is aware of the publications quoted by the MS and it notes that they have been taken into consideration when it has assessed the allergenicity of the single events and the new proteins expressed in the single events. The Panel is not aware of any new information that would change its opinion. In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would in particular impact on the allergenicity.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			possibility that the protein can enter the respiratory tract after exposure to e.g. mill dust. References: Moreno-Fierros L, Ruiz-Medina EJ, Esquivel R, López-Revilla R, Piña-Cruz S., 2003. Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand J Immunol., 57: 45-55. Prasad S.S.S.V. & Shethna, Y.I., 1975. Enhancement of immune response by the proteinaceous crystal of Bacillus thuringiensis var thuringiensis. Biochem Biophys Res Commun., 62: 517-521. Rojas-Hernández S, Rodríguez-Monroy MA, López-Revilla R, Reséndiz-Albor AA, Moreno-Fierros L., 2004. Intranasal coadministration of the Cry1Ac protoxin with amoebal lysates increases protection against Naegleria fowleri meningoencephalitis. Infect Immun., 72:4368-4375 Vazquez-Padron RI. Martinez-Gil AF. Ayra-Pardo C. Gonzalez-Cabrera J. Prieto-Samsonov DL. de la Riva GA., 1998. Biochemical characterization of the third domain from Bacillus thuringiensis Cry1A toxins. Biochem Mol Biol Int., 45(5):1011-20. Vazquez RI. Moreno-Fierros L. Neri-Bazan L. De La Riva GA. Lopez-Revilla R., 1999. Bacillus thuringensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol., 49: 578-84. Vazquez-Padron RI. Gonzales-Cabrera J. Garcia-Tovar C. Neri-Bazan L. Lopez-Revilla R. Hernandez M. Moreno-Fierro L. de la Riva GA., 2000a. Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun., 271:54-8.	
Spain	Ministry of the Environment	General comments | D, 02 Information on the sequences actually inserted or deleted | D, 07 Information on any toxic, allergenic or other harmful effects	SPANISH COMMENTS EFSA/GMO/UK/2005/21: 59122 x 1507 x NK603 MAIZE Comments of the National Commission on Biosafety of Spain General comments On the other hand, due to the fact that a specific analytical method for the detection and quantification of this hybrid has not been provided yet, Spain is concerned about the legal and administrative implications which could come out	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
		on human or… | D, 07.08 Toxicology | D, 12 Environmental Monitoring Plan | D, 12.03 General Surveillance of the impact of the GM plant	from the analytical results in the final product in order to identify and quantify the parental lines (59122, 1507 and NK603) and the hybrid (59122 x 1507 x NK603) separately. D.02. Information on the sequences actually inserted or deleted The notifier should submit the chromosomic location of the inserts, and the complete sequence of the inserts (pointing out the encoding reading phases, and the control elements of the expression), corresponding to the three parentals and the hybrid. In some Southern blot analyses it is not use copy controls, so it is not possible determinate properly the copy number of the insert. In some cases three copies seem have been inserted. D.7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food and feed D.07.09 - Toxicology. Toxicity studies with combined proteins should be presented, because of the fact that the whole consumption of the proteins could involve toxic effects different to separately administration. D.12. Monitoring Plan D.12.03 - General Surveillance of the impact of the GM plant. The consent holder should provide further details of the arrangements of the monitoring plan, in particular for general surveillance, indicating which existing network programs could be used, the type of information that should be collected and a more detailed monitoring methodology in order to have a monitoring plan which could be implemented in a harmonised manner among the importer Member States.	For all three events the sequence of the insert and flanking regions are supplied in the original dossiers of the parental lines. According to the guidance of stacked events, only the intactness of the events should be confirmed in the hybrid to confirm the earlier perfomed safety assessment. Additional information has been requested on the intactness of the inserts and the flanks. Molecular equivalence of the 59122, 1507 and NK603 insert in the hybrid was determined by Southern analysis, using *Sac*I, *Hind*III and *EcoR*V digested genomic DNA and probes of the *pat*, *Cry*1F, *Cry34*Ab1, *Cry35*Ab1 and CP4 *epsps* genes. From the hybridisation patterns of 59122x1507xNK603 and all parental lines, it was concluded that the organisation of sequences in the insert are unchanged. Also the intactness of the 1507 insert and of the 3' side of the 59122 was confirmed. Additional information has been supplied on the intactness of the NK603 insert and the 5' of the 59122 insert in the hybrid line. The intactness of the NK603 insert was demonstrated by Southern analysis of *Msc*I and *Sca*I-digested DNA. The intactness 59122 insert in the hybrid line was confirmed by results obtained by event-specific real time has been requested regarding intactness of the events. The data as supplied in the application demonstrate the presence of only one copy of each event. The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided

Country	Organisation	Reference	Comment	EFSA GMO Panel response
				further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion
The Netherlands	Ministry of Agriculture, Nature and Food Quality and Ministry of Health	D, 07.01 Comparative assessment	Upon comparison of the stacked transgenic maize versus non-transgenic control maize, statistically significant differences have been found for many compositional parameters. Whilst these differences fall within the wider background ranges obtained from literature and the ILSI crop composition database, the report does not contain a comparison with the compositional ranges of the transgenic "single events" 59122, 1507 and NK603. To facilitate the interpretation of the observed differences, the applicant should provide compositional data on the transgenic "single events". In addition, a presentation of the amino acid composition expressed as % of total amino acids instead of % dry weight should be provided, in order to facilitate the comparison of maize samples with different protein contents.	See section 4.1.2 and 4.1.3
The Netherlands	Ministry of Agriculture, Nature and Food Quality and Ministry of Health	D, 12.03 General Surveillance of the impact of the GM plant	The Dutch CA under the 2001/18/EC has the following procedural point: A general surveillance plan is supplied. The applicant makes a distinction between reporting direct and indirect effects in the monitoring plan. According to the applicant direct effects will be reported annually and indirect effects only at the stage of re-evaluation or at the end of a given consent. The Dutch CA under the 2001/18/EC is of the opinion that the applicant should report unexpected direct and indirect effects annually.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance. Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided. See section 6.1.3 of the scientific opinion. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan (on an annual basis).

Application EFSA-GMO-UK-2005-21 (Maize 59122x1507xNK603) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**