Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **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 for 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.	Outside the remit of the GMO Panel.
Austria	Ministry for Health, Family and Youth	General comments	Concerning the single events of this notification, Austria is still of the opinion that their risk assessment with regard to e.g. agronomic traits, compositional analysis, 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 stacked event before clarifying the shortcomings of the single events.	The GMO Panel cannot influence when applicants makes an application. In this application, the applicant bases its safety assessment on the safety of both parental maize events. Thus, both parental events need to have an opinion from the GMO-Panel before this application on the stacked event is given an opinion.
Austria	Ministry for Health, Family and Youth	General comments	Labelling If the notifier will not be the operator, the company shall ensure that all labelling requirements are transmitted to and fullfilled by the operator.	Outside the remit of the GMO Panel.
Austria	Ministry for Health, Family and Youth	D, 07.01 Comparative assessment	The field trials were conducted at 5 locations in Argentina in 2004/2005 according to Production Plan 04-05-50-01 (Bader 2006). No information about pestizide application was given. 15 commercial maize samples were used as reference data set and 9 analytes were tested in forage and 52 in grain. Significant differences for the GM grain: • higher contents of stearic acid (mean and 3 sites) • higher contents of total fat (mean and 2 sites) • lower contents of Vit. B2 (mean and 1 site) • lower contents of ADF (mean only) • higher contents of p-coumaric acid (1 site) Forage: • higher contents of ash (mean only) • lower contents of total fat (mean only) • higher contents of NDF (2 sites) With regard to these findings, clarification is needed. Like in previous reports it is suggested that to focus on compound relations and interactions, reflecting the plant´s physiological status much more precisely than lists of analytical data alone; this would give a better assessment of substantial equivalence. In previous reports the C/N	The Panel notes these comments and asked the applicant to provide clarification on the pesticide treatments during the field trial. The requested information was obtained. The comparative compositional assessment as part of the safety assessment is described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. Regarding the assessment, see the opinion.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			ratio of the GM maize was found to differ strongly from the control line (3272, NK603, MON59122, 1507, MON59122xNK603 and MON59122x1507xNK603). In this case no such difference was found, although the parental line NK603 showed this difference in previous field studies. Since the C/N ratio is strongly influenced by agricultural applications it would be interesting to know whether herbicides have been used or not. Without this information it is impossible to draw any conclusions. But similarily to MON 89034 there were clear differences in the fatty acid contents. A comprehensive survey of fatty acid contents in corn hybrids showed a wide range of fatty acid profiles as well as small but significant correlations among protein, starch and the amounts of several fatty acids [Dunlap F.G., White P.J., Pollack L.M., Brumm T.J. (1995): Fatty acid composition of oil from adapted, elite corn breeding materials. Journal of the American Oils Chemists´ Society, Vol. 72/9; pp. 981-987]. 14 analytes were significantly different only in one site and not in the combined sites and are therefore not considered biologically relevant. In the applications it is generally stated, that differences either are not consistent over the sites or/and are within the range of historical values and have therefore no biological relevance. But this assessment does not consider the site influences on the GM corn as compared to the site influences on its near genetic comparator in detail which should be the focus of comparative field trials, since in future the performance of the newly developed GM corn in a wide range of sites and climatic conditions is relevant to its agronomic success. There might be no trend in the content comparisons between sites, but any significant difference could reflect plant/site effects that need to be investigated to obtain a deeper understanding of the GM plant´s behaviour and adaptability to differing growing conditions.	

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry for Health, Family and Youth	D, 07.04 Agronomic traits	The field trials were conducted at 5 sites in Argentina 2004/5. Seedling vigor was significantly lower in only 1 (B1) of the 5 sites (4,3 vs 5,0). Comparing all data of seedling vigor shows that in this site the seedling vigor of the control maize was also very low as compared to the other sites. This could be an indication that the GM maize is possibly more affected in less than optimal growing conditions. In another site (B3) the root lodging was much higher as compared to the other sites and it has been noted, that the GM MON89034xNK603 had more root lodged plants than the control (25 vs 21,7 n.s.). Since root lodging is generally associated with environmental factors such as heavy rains with wind it could indicate an inferior adaptation capability of the GM maize to adverse weather conditions. In a similar way in the field trial with MON89034 maize in the USA 2005 due to dry weather and a storm the number of stalk lodged GM MON89034 plants was double the number of the control (Kendrick and Clark 2006a; Table 5). This could again point to a potential weakness of the GM plants grown in drier than normal conditions when exposed to an abiotic stressor such as a storm. Under better weather conditions the field trials of 2004 showed less stalk lodging in the GM 89043 plants. Under optimal growing conditions (SF site) the GM stacked maize revealed the same performance like the control and had a significantly higher yield. Although even here wind damage was higher in the GM plants (Table 12). This shows that it is necessary to pay more attention to the interactions between growing and site conditions and crop performance to evaluate the adaptability of the test crop. The combined site analyses give no information of site/plant interactions. Furthermore at least 2 years of cultivation would provide a safer basis of assessment. For a sustainable and balanced crop performance a high adaptability is preferable.	The GMO Panel has carefully considered the compositional data in the application. This application only addresses the stacked events in maize MON 89034 x NK603 and not the single parental events. Opinions on the single events maize MON 89034 and maize NK 603 have already been given by the Panel. Recommendations for the applicant when preparing their application is obtained in the "Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed" and in the "Risk assessment of plants containing genetic modification events combined by crossing". These recommendations could be interpreted as minimal requirements. The scope of the application is for food and feed uses, import and processing of maize MON98034 x NK603 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize MON89034 x NK603.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry for Health, Family and Youth	D, 07.08 Toxicology	Whole feed toxicity studies Concerning maize NK603: 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) was conducted. 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. Statistically significant differences between the GM-test- and control groups: • Body weight gain was generally higher in the GM-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. • Feed intake was generally higher in the GM-test group, some differences were significant. • Elevated levels of MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) in the GM-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 in the GM-test group. • In the GM-test group the clinical chemical parameters 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 in the GM-test group. Unfortunately kidney weights were not included. Concerning the stacked event No whole feed toxicity study was performed with the	No 90-day toxicity study in rats was included in the application.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
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			stacked event – this can not be regarded as state of the art.	
Austria	Ministry for Health, Family and Youth	D, 07.08 Toxicology	Rapid digestion in simulated gastric fluid No rapid digestion studies in simulated gastric fluid with the stacked event were performed. Furthermore no risk assessment on the fate of Cry1A.105 or Cry2Ab2 other than E.coli produced novel proteins was conducted. Testing the whole corn in digestion studies might differ in findings as the kernel might protect the novel protein during the passage. Oral toxicity studies and repeated oral toxicity tests No studies of oral toxicity and repeated oral toxicity tests were conducted with the stacked event. It is suggested to carry out these studies in order to complete the risk assessment.	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed by the GMO-Panel and found to be as safe as conventional maize varieties. A study showed that maize MON 89034 x NK603 is as nutritionally wholesome for broiler chickens as conventional maize. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional animal feeding study is needed.
Austria	Ministry for Health, Family and Youth	D, 07.08 Toxicology	Whole feed toxicity studies Concerning maize 89034 A 90-day feeding study in rats with MON 89034 has been conducted (Kirkpatrik 2007). Similarly, for the 90-day-feeding study in rats the characterisation of the control line is incomplete. The notifier states that the control substance was a conventional variety of corn with background genetics comparable to the test substance that does not produce the Cry1A.105 or Cry2Ab2 proteins. In Appendix D p. 446, only verification of diet identity of Cry2Ab2 is present, no such verification of diet identity of Cry1A.105 could be found. Full reference with respect to the breeding history should be given. With respect to the analysis of potential GM contaminations in the control and test substance, the notifier provides a certificate of analysis stating that contamination of MON89034, NK603 and MON89597 was checked (see Kirkpatrick 2007). This certificate indicates negative results, however it does not state which limit of detection is referred to. There was a slight elevation of body weight in males of the 11% GM diet during the first week. No other significant weight differences were found. But in males and females the body	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed by the GMO-Panel and found to be as safe as conventional maize varieties. No 90-day toxicity study in rats was included in the present application regarding maize MON 89034 x NK603.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			weight curve is marginally but consistently above the control curve (Fig. 1 and 2). In hematology some inconsistent findings were seen in the platelet count reduced in females of the 11% GM group. This difference is not considered biologically meaningful because it was not seen in the 33% GM group and no alterations in corpuscular compounds of the blood were seen. Additionally there were differences in the relative thyroid/parathyroid weight only in females of the 33% GM group. No changes in metabolism or histological findings could corroborate any health disturbance. But in females of the 33% GM group urinar calculi occurred and in the same group microscopic investigations reveiled chronic progressive necropathy and transitional hyperplasia, which have not been regarded as biologically meaningful or relevant. Since single changes in kidney charactersitics were reported also in other studies, the kidney might be an organ sensitive for GM effects. At this point these findings can not be classified but in a first approach the kidneys could be suggested as an organ of special interrest in investigating possible adverse GM effects.	
Austria	Ministry for Health, Family and Youth	D, 07.08 Toxicology	Whole feed conversion studies A 42 day broiler study has been conducted with the stacked event MON 89034xNK603 (Davis 2006). 8 diets were compared, only one contained the stacked event. Thus 700 animals were fed with non-GM diets and only 100 with the test diet. The inclusion of 6 commercial diets in the comparison between GM corn and its isogenic control is not regarded as appropriate. A new broiler study based on a more suitable test design should be carried out. Like in the broiler study with MON 89034 the feed was pelleted, involving protein changing applications (steam heat and pressure). This should be avoided in comparative feeding studies, where protein effects and quality are the focus of the investigation. Additionally it has to be mentioned that weight data on inner organs such as liver and kidney could give easily	The GMO Panel has no problem with the design of the 42-day feeding study in broiler chickens; which is a study performed to assess the nutritional quality of the test material. The result of the study shows that maize MON 89034 x NK603 is as nutritionally wholesome as conventional maize.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			attainable additional information. It is therefore suggested to include these in future agronomic trials.	
Austria	Ministry for Health, Family and Youth	D, 07.09 Allergenicity	Allergenicity No allergenicity study with the stacked event of was performed - neglecting potential interactions between the transgenes. Therefore it is suggested to carry out these tests in order to ensure a comprehensive risk assessment. It must be stated again, that comprehensive risk assessment as described in Spök et al. [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.] should be carried out. The recommendations given for a standardized and harmonized approach to the generation, presentation and interpretation of data concerning allergenicity of GM products are based on in depth scientific studies, performed by experienced scientists in the field. The proposed tests should be performed by the notifier and the resulting data provided in order to guarantee a high level of safety and public confidence in the approach taken.	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential allergenicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. Expression data was obtained from material collected from field trials at five sites in Argentina in 2004. Originally the expression data was not presented per site and tissue studied, but only as average expression in the different tissues across all sites combined. However, the applicant supplied expression data for each tissue and each trial site on request from the Panel. These data showed that the expression of Cry1A.105, Cry2Ab2 and CP4 EPSPS is comparable in maize MON 89034 x NK603, MON 89034 and NK603, respectively. The panel is of the view that no additional study is needed.
Austria	Ministry for Health, Family and Youth	D, 08 Post-market monitoring of GM food/feed	According to Art. 5 (3) k) of EU-Regulation 1829/2003 a post-market monitoring-plan should be added to the dossier.	This Article of the EU Regulation 1829/2003 refers to the post-market environmental monitoring plan, and not to post-market monitoring of GM food and/or feed.
Austria	Ministry for Health, Family and Youth	D, 12 Environmental Monitoring Plan	Case-specific Monitoring The notifier states that maize MON89034xNK603 can accidentally be introduced into the environment (“icidental spillage”, “environmental release would be more likely to occur during import, storage and processing”,...). However, surveillance or management systems which are suitable to monitor and detect possible	The EFSA 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.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			unintended environmental exposure by accidental spillage or release of this maize have not been suggested by the notifier (see p.120, monitoring plan). Also no measures are taken to ensure that the reporting of unintended environmental release will be carried out by the relevant stakeholders involved. In order to cover the risk of accidental spillage or unintended release into the environment of GM maize MON89034xNK603 a case-specific monitoring plan should be provided. General surveillance The general surveillance plan proposed by the notifier is limited to providing information and creating awareness among stakeholders and to collect information via key networks, stakeholders and observation programmes. It remains unclear which specific networks will be informed within the individual member states and how it will be ensured that the information would reach the relevant stakeholders. A more specific general surveillance plan should be set up with respect to the geographical area and contact points, institutions or networks used. Details must be provided on the information networks used and the evidence that these surveillance networks actually collect the relevant information in the individual member states and that they have agreed to make any information available on general surveillance of the product. In summary, the information provided by the notifer is considered to be too general and too imprecise for a surveillance plan of unintended effects on human or animal health and the environment. The plan should be fundamentally revised.	The information initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See the scientific opinion
Belgium	Belgian Biosafety Advisory Council	A. General information	As MON 89034 × NK603 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 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 MON 89034 ×	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			NK603 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.	maize lines. The panel is of the view that no studies on developmental toxicity and teratogenicity is needed.
Belgium	Belgian Biosafety Advisory Council	D, 02 Information on the sequences actually inserted or deleted	1.On which generation of MON 89034 x NK603 hybrid/inbred has the genomic DNA been extracted? This is an important issue concerning the genetic stability (see D5). 2.It is mentioned that both inserts are on separate chromosomes in the nuclear genome. I do not remember any data showing or commenting on which chromosome the inserts are found in the parent lines. A precise reference of this information should be given. 3.Because it is considered that there is a low likelihood of molecular interactions between the inserts, the applicants did not start again a complete molecular analysis to demonstrate the size, copy number and integrity of the 2 inserts. Only two Southern blot analysis were performed and showed that the size of the inserts and flanking regions correspond to those of their respective parents. The size of the bands obtained in the control lanes including plasmid DNA cannot be understood from the technical dossier itself, but a detailed description of the Southern blot experiments is found in Tian et al., 2006. On which generation of MON 89034 x NK603 hybrid/inbred has the genomic DNA been extracted? This is an important issue concerning the genetic stability (see D5).	According to the EFSA guidance document on stacked events (EFSA, 2007): “Comparisons between the insert structures in the original events and the GM stacks should be carried out on materials representative of those designed for commercial production, i.e. which will enter the environment and the food/feed chain.” In this case, F1 seed material is commercialised.
Belgium	Belgian Biosafety Advisory Council	D, 02 Information on the sequences actually inserted or deleted	Attention : correction for comment 2 under D, 02 2. It is mentioned that both inserts are on separate chromosomes in the nuclear genome. A precise reference of the data showing on which chromosome the inserts are found in the parent lines should be given.	The stable inheritance of both MON 89034 and NK603 events have been demonstrated earlier. Both events are intact in the MON 89034 x NK603 hybrid. Chromosomal locations of the inserts are not relevant to the safety of this product.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **ANNEX G** **Comments and opinions submitted by Member States during the three-month consultation period**				
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Comments from National Competent Authorities under Directive 2001/18/EC				
Belgium	Belgian Biosafety Advisory Council	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	The genetic stability of the insert was not tested. The applicants justified this by theoretical arguments based on previous studies on recombinations and concluded that it is appropriate to apply results of the characterisation performed on the parental lines MON 89034 and NK603. Even though all the data support very unlikely recombination events, it the demonstration of genetic stability of the inserts in the marketed grains and in subsequent generations (which will be consumed as food or feed) would be useful and fit with the guidelines for the safety assessment of genetically modified crops for food and feed use. Parts of the two T-DNA inserts contain homologous sequences, and Hsp70 intron and TS-SSSU-CTP DNA are maize sequences that could potentially recombine with endogenous DNA. The applicant is invited to comment on possible co-silencing effects in this context. This is in line with the EFSA guidance document on stacked events (EFSA Journal, 2007, 512, 1-5)	There are no indications from comparative agronomic performance and compositional analyses of any unintended effect caused by the combination of the single events in the hybrid.
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	1. Cry1A.105, Cry2Ab2 (both MON 89034) and CP4 EPSPS (NK603) proteins were tested in earlier studies. These studies showed no evidence of acute toxicity. Further testing of these proteins for acute toxicity is not required. 2. But the proteins made by the inserted genes were tested (in chronic studies) separately and not together: this does not offer the opportunity to have data of possible interactions between these proteins. 3. See dossier 2007/37 for a remark on potential endocrinal disruption of substances with a pesticide action.	See section 5.1.3. of the opinion. As far as the GMO panel is informed, Cry proteins and CP4 EPSPS, and the pesticide residues produced as a result of the action of the later protein on glyphosate sprayed maize, have not been identified as endocrine disruptors.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
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Comments from National Competent Authorities under Directive 2001/18/EC				
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Testing of the whole GM food/feed 1. Broiler performance According to tables 4, and 5 the Cry1A.105 and Cry2A2 protein content is similar in MON 89034 and MON 89034 x NK603 grain. According to table 6 the CP4 EPSPS protein content is similar in MON 89034 x NK603 and NK603 grain. The toxicity study should report the weights of organs like kidneys and liver that are the first to be affected by toxins. Consumers usually consume corn for a long period of time so there is a need of a chronic toxicity study. 2. 13-Week feeding study in rats. This study should be performed since synergistic effects of the proteins under investigation cannot be excluded beforehand. Furthermore, these results could have helped in deciding whether the problems, which arose during the 13-week feeding study in rats with MON 89034 (see comment for dossier EFSA/GMO/NL/2007/37), were of importance or simply due to chance.	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional animal studies are needed. The 42-day feeding study in broiler chicken is seen by the panel as a study on nutritional adequacy. To obtain more data from this study the Panel requested the applicant to report the data of the broiler chicken feeding study for each sex separately. The requested data identified no concern regarding the wholesomeness of maize MON 89034 x NK603 as food and feed.
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	1. General comments: see dossier 2007/37 2. For the allergenicity evaluation of Cry1A.105 and Cry2Ab2: see commnents of the Belgian experts in dossier 2007/37. 3. For the CP4 EPSPS protein, a 30 % homology was found with the Dermatophagoides farinae 2 protein (Der f 2). Although this homology is under the limit of 35 %, it would be interesting to compare the 3d structures of Der p 2 and CP4 EPSPS and to test some sera of patients allergic to Der p 2. 4. Assessment of allergenicity of the whole GM plant. This has not been evaluated in the application. As in the comments for application **EFSA/GMO/NL/2007/37**, the reviewer wishes to emphasize that the rationale of this section is to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source. Although not frequent, food allergy to maize has been described and major allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002). In addition,	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential allergenicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional study is needed.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) 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				
			other potential allergens have been detected (Weichel et al. 2006). The introduction in the plant of Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins, even if not allergenic, might interfere with the expression levels of other maize proteins, including allergens. Care must be taken that food allergy to maize grain does not become more frequent due to the introduction of new traits and the interferences thereof. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified MON89034 x NK603 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out. Pasini et al. (2002) IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. Allergy, 57:98-106 Pastorello et al. (2003) Lipid-transfer protein is the major maize allergen maintaining IgE-binding activity after cooking at 100 degrees C, as demonstrated in anaphylactic patients and patients with positive double-blind, placebo-controlled food challenge results.J Allergy Clin Immunol, 112;775-83 Weichel et al. (2006) Screening the allergenic repertoires of wheat and maize with sera from double-blind, placebo-controlled food challenge positive patients. Allergy, 61:128-35.	
Belgium	Belgian Biosafety Advisory Council	D, 08 Post-market monitoring of GM food/feed	As no long term toxicity studies has been done, it is not possible to exclude long term effect of GMO consumption. That's why it is required to do a follow-up of the GM food post-market	No potential risk has been identified that needs to be followed up by post-market monitoring of food and feed from maize MON 89034 x NK603.
Belgium	Belgian Biosafety Advisory Council	D, 12.01 General	See comments for dossier 2007/37	See response provided for application EFSA/GMO/NL/2007/37

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **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				
Denmark	Danish Environmental Protection Agency	D, 02 Information on the sequences actually inserted or deleted	Ad D.2.b The applicant has sequenced and about 600 bp outside the inserted DNA and identified it as being maize DNA. We would like to know whether homology search to known maize genome DNA gave indication of potential genes in this area that might have been influenced by the insertion. Ad D 11.4.4 Reporting of indirect or delayed effect aught to be given in the annual reports on the general surveillance of the MON89034 Mays.	Regarding the molecular characterisation of the MON 89034 event, this information is detailed in EFSA's scientific opinion on MON 89034 maize (EFSA, 2008). The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 6.1.3 of the scientific opinion
Denmark	Danish Environmental Protection Agency	D, 02 Information on the sequences actually inserted or deleted	Ad D.2.b The applicant has sequenced and about 600 bp outside the inserted DNA and identified it as being maize DNA. We would like to know whether homology search to known maize genome DNA gave indication of potential genes in this area that might have been influenced by the insertion. Ad D 11.4.4 Reporting of indirect or delayed effect aught to be given in the annual reports on the general surveillance of the MON89034 Mays.	Copy of question above
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 applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA,

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 5.2.2 of the scientific opinion
France	MINEFE / DGCCRF	D, 07.08 Toxicology	(7.8.2) Etude de la toxicité subchronique Aucune étude de toxicité subchronique n'a été réalisée chez le rat avec le maïs hybride MON 89034 x NK 603. Il est noté que : - aucun effet toxique ou délétère chez l'animal de laboratoire n'a été mis en évidence pour les 3 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, - l'étude d'alimentarité réalisée chez le poulet permet de conclure à l'équivalence nutritionnelle du maïs hybride avec son témoin, - une étude de toxicité subchronique de 90 jours réalisée chez le rat avec le maïs parental NK 603 n'a montré aucun effet délétère chez l'animal, - une étude de toxicité subchronique de 90 jours a été réalisée chez le rat avec le maïs parental MON 89034 mais que dans cette étude, des altérations histologiques rénales son	Both parental genetically modified events, NK603 and MON 89034, respectively, and the proteins expressed in these maize events (Cry1A.105, Cry2Ab2 and two versions of CP4 EPSPS) have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties (see previous opinions of the GMO Panel). The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional animal feeding studies are needed. Furthermore, the parental maize events MON 89034 and NK603 were included in field trials. The harvested materials (different tissues) of these parental events, the stacked maize MON

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			mentionnées qui nécessitent d'apporter des explications complémentaires sur la différence d'apparition des calculs dans la vessie entre les données historiques (0,49 %) et l'incidence de 10 % observée au cours de cette étude chez les animaux femelles du groupe à la forte dose de MON 89034. En l'absence de ces explications complémentaires, il n'est pas possible, de considérer que ces éléments sont suffisants pour démontrer la non toxicité des produits dérivés de l'hybride MON 89034 x NK 603. Une étude de toxicité 90 jours réalisée avec un maïs hybride portant ces événements de transformation aurait permis de s'assurer que les altérations histologiques rénales mentionnées n'étaient pas liées à la modification génétique introduite dans le maïs MON 89034. INTERNET TRANSLATION : (7.8.2) Etude of the poisonousness subchronique No study of poisonousness subchronique was realized with the rat with the corn crosses MY 89034 x's NK 603. It is noted that: - no toxic or harmful effect with the laboratory animal was made obvious for the 3 proteins of interest, - the levels of expression of the proteins of interest, considering the typical observed gaps, not being modified with the crosses compared to the levels measured with the parents, such a element is in favor of an absence of interaction between the transformation events, - the study corn crosses with his witness, - a study of poisonousness subchronique of 90 realized days with the rat with the parental corn NK 603 did not show any harmful effect with the animal, - a study of poisonousness subchronique of 90 days was realized with the rat with the parental corn MY 89034 but that in this study, kidney histological deteriorations his mentioned ones that necessitate to bring of the The difference of appearance of the calculations in the vessie between the historic data (0,49%) and the incidence of 10% observed one during this study with the female animals of the group to the strong dose of MY 89034. In the absence of these	89034 x NK603 and an appropriate non-GM control was used to study the expression of the transgenes. On request from the Panel, the applicant supplied expression data for each individual trial site and sample. The GMO Panel concluded that the expression in the stacked event was comparable to the expression in the single parental events.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			supplementary explanations, it is not possible, to consider that these elements are sufficient to show the non-poisonousness of the diverted products of the crosses MY 89034 x's NK 603. A poisonousness study 90 realized days with a hybrid corn carrying these transformation events would have allowed assuring itself that histological kidney mentioned deteriorations were not linked to the genetic modification introduced in the corn MY 89034.	
France	MINEFE / DGCCRF	General comments	En conclusion, l'Agence française de sécurité sanitaire des aliments considère que, compte tenu des réserves faites sur l'étude de toxicité 90 jours avec le maïs MON 89034, elle ne peut pas se prononcer sur la sécurité sanitaire des produits dérivés des variétés de maïs portant l'événement de transformation MON 89034 x NK 603. L'Afssa rappelle également la nécessité de fournir des informations complémentaires qui permettent de lever toute ambiguïté sur le fait que **l'intégration de l'évènement MON 89034 s'est faite dans une région fonctionnelle ou non du génome du maïs**. INTERNET TRANSLATION : In conclusion, the French Agency of sanitary security of the foods considers that, considering the reserves done on the poisonousness study 90 days with the corn MY 89034, she cannot pronounce itself on the sanitary security of the diverted products of the varieties of corn carrying the transformation event MY 89034 x's NK 603. The Afssa recalls equally it necessitated to furnish supplementary the news that allow getting up all ambiguity on the Fact that the integration of the event MY 89034 was done in a functional or not region of the genome of the corn.	The safety of both parental maize events, MON 89034 and NK603, have been adressed in earlier opinions of the GMO Panel related to the parental events. The GMO Panel has not identified any safety issue that motivates the requirement of 90-day feeding study in rodents.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	General comments	The Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion that further information is required to conclude on the risk assessment of dossier EFSA/GMO/NL/2007/38	See Conclusions and Recommendations of the scientific opinion
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. Seeds and products derived from MON 89034 x NK603 should be accompanied by an instruction leaflet including the information that MON 89034 x NK603 has not been approved for cultivation.	Outside the remit of the 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 Cry1A.105, Cry2Ab2, and CP4 EPSPS proteins were obtained from field trials at five locations in Argentina in one growing season. Samples from several tissues and different growth stages were analysed. The results indicate, that the expression of the analysed proteins is essentially similar to the expression in the parental lines. Separate data from single locations were not presented by the applicant. For a thorough assessment of the variability/span of protein expressionlevels further evaluation is needed. The applicant is requested to provide a site specific analysis of data. The presentation of data over more than one growing seasons including sufficient statistics would be advisable.	Expression data is obtained from material collected from field trials at five sites in Argentina in 2004. Originally the expression data was not presented per site and tissue studied, but only as average expression in the different tissues across all sites combined. However, the applicant supplied expression data for each tissue and each trial site on request from the Panel. According to the EFSA guidance document on stacked events (EFSA, 2007): “For the stacked events at least one year of field trial data is required”. The data provided on protein expression in the single events and in the hybrid do not raise any safety concern.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.01 Comparative assessment	The compositional analysis is based on grain and forage material sampled from field trials at five locations in Argentina in one growing season. The identified compositional differences, including the statistically significant, of MON 89034 x NK603 maize compared to conventional maize do not cause concerns with respect to	Data on individual field trial sites is presented in Drury et al. (2006). In the document “Risk assessment of plants containing genetic modification events combined by crossing” the GMO-Panel state: “Where the

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			food and feed quality and safety. However, the supplied studies do not detail information on individual locations and respective statistics. These data should be supplied by the applicant. In accordance with EFSA Guidance Document (2004) sampling of material over more than one season per location would be advisable. 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 both blocks of genetically modified plants exposed to the intended herbicide and blocks not exposed to the herbicide". In the field experiment (Drury et al. 2006), it is not indicated whether MON 89034 x NK603 maize plots were treated with the intended herbicide, i.e. glyphosate ("samples at the field sites were grown under normal agronomic field conditions"). An explanation by the applicant why the experimental design does not include test plots with differing herbicide treatment should be requested.	substantial equivalence of parental material containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one years filed trialling of events combined by crossing is acceptable where geographical localities are representatie of the climatic conditions to which such crops will be exposed. Based on the outcome of this assessment additional follow-up analysis of compositional characteristics over further growning seasons may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may trigger further assessment." The GMO Panel agree that in the original application it was not apparent what pesticide treatments were performed at each field trial site. This information was provided by the applicant on request from the Panel.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.08 Toxicology	Details regarding toxicology of Cry1A.105 and Cry2Ab2 Proteins are given in the corresponding section of application **EFSA/GMO/NL/2007/37**. Therefore the Federal Office of Consumer Protection and Food Safety refers to its statement on application EFSA/GMO/NL/2007/37.	See response provided for application EFSA/GMO/NL/2007/37
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.09 Allergenicity	Details regarding allergenicity of Cry1A.105 and Cry2Ab2 Proteins are given in the corresponding section of application **EFSA/GMO/NL/2007/37**. Therefore the Federal Office of Consumer Protection and Food Safety refers to its statement on application EFSA/GMO/NL/2007/37.	See response provided for application EFSA/GMO/NL/2007/37
Germany	Federal Office of Consumer Protection	D, 12 Environmental Monitoring	The general surveillance plan is basically acceptable, but needs some specifications. As part of the "active surveillance" it is planned to inform traders and processors	The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA,

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
	and Food Safety (BVL)	Plan	as well as to gather data from different communication networks. It is requested that the applicant specifies in detail, how and which data will be queried and gathered. The use of questionnaires could be appropriate measures to survey this data. In addition, it might be useful to integrate food and feed surveillance in coordination with the competent authorities. Information about the use of this 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. Submitting monitoring reports on an annual basis is sufficient.	2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion
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 a substantial equivalence of MON 89034 x NK603 maize and conventional maize based on this information are premature. Due to indications for possible adverse effects detected in a 90-day feeding study in rats with MON 89034 maize (see application EFSA/GMO/NL/2007/37), the applicant is asked to provide a subchronical feeding study in mammals with MON 89034 x NK603 maize. Although application	The Panel is of the view that no additional data on protein expression, chemical composition and agronomic and phenotypic characters is needed. Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			EFSA/GMO/NL/2007/38 does not include the cultivation of MON 89034 x NK603 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). With regard to references in application EFSA/GMO/NL/2007/38 to application EFSA/GMO/NL/2007/37 for authorisation of MON 89034 maize for all food and feed uses in the EU in accordance with Articles 3(1) and 15(1) of Regulation (EC) No 1829/2003 and for import and processing of MON 89034 maize in the EU in accordance with Part C of Directive 2001/18/EC maize, we refer to the statement of the German Competent Authorities including comments of the Federal Agency for Nature Conservation on application EFSA/GMO/NL/2007/37.	of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional animal studies are needed. The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the 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 this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).*

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				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 MON 89034 x NK603 maize, a more robust and reliable data basis is required, including a higher 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 give a rough estimate of expression levels only. 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. Values for the expression of Cry1A.105 and Cry2Ab2 in some tissues differ considerably between the single study sites. This suggests that stability of the expression in MON 89034 x NK603 maize depends on site-specific ecological factors. Hence, a thorough statistical analysis comparing expression values at the different sites should be provided to address this suggestion. The applicant is asked to provide a statistical analysis comparing expression values from MON 89034 x NK603	According to the EFSA guidance document on stacked events (EFSA, 2007): "For the stacked events at least one year of field trial data is required". The data provided on protein expression in the single events and in the hybrid do not raise any safety concern. Expression data is obtained from material collected from field trials at five sites in Argentina in 2004. Originally the expression data was not presented per site and tissue studied, but only as average expression in the different tissues across all sites combined. However, the applicant supplied expression data for each tissue and each trial site on request from the Panel. The requirement of the applicant is partly defined in the document "Risk assessment of plants containing genetic modification events combined by crossing". Based on this guidance and other considerations the Panel accepted the supplied information on how the GM plant differs from the recipient plant in agronomic, phenotypic and ecological characteristics.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			maize with expression values in MON 89034 maize and NK603 maize.	
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 application EFSA/GMO/NL/2007/38 (see included study by Phillips at al. 2006) do not indicate a potential for differences in reproduction, dissemination and survivability of MON 89034 x NK603 maize, the selected parameters themselves cannot sufficiently indicate such changes. Data presented on disease incidence and insect damage are of limited value because pesticides were applied to all plots. Moreover, 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 is not considered sufficient to support the conclusion of a substantial equivalence of MON 89034 x NK603 maize and conventional maize, which is the basis of further conclusions in application EFSA/GMO/NL/2007/38. 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 frost tolerance, seed dormancy, or competitiveness of MON 89034 x NK603 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	The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize MON89034 x NK603. The requirement of the applicant is partly defined in the document "Risk assessment of plants containing genetic modification events combined by crossing". Based on this guidance and other considerations the Panel accepted the supplied information on how the GM plant differs from the recipient plant in agronomic, phenotypic and ecological characteristics. The Panel also notes the scope of the present application, which is food and feed uses of maize MON 89034 x NK603, import and processing. The Panel also notes that the parental maize was included in the field trials. Different materials of maize 89034 x NK603, maize MON 89034, maize NK603, and the non-GM control to maize 89034 x NK603 were studied for the expression of the newly expressed proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS. Expression levels were found to be comparable in the parental and stacked GM events.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			between-site variation of the data. According to the EFSA Guidance Document for the risk assessment 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 provide a statistical analysis comparing data from MON 89034 x NK603 maize with data from MON 89034 maize and NK603 maize. To include possible effects of ecological factors in such a comparison, the applicant is asked to provide a study where the parental GM lines MON 89034 maize and NK603 maize are included in the study design at the same study sites.	
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 is not considered sufficient to support the conclusion of a substantial equivalence of MON 89034 x NK603 maize and conventional maize, which is the basis of further conclusions in application EFSA/GMO/NL/2007/38. 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. The applicant is asked to provide composition data from MON 89034 x NK603 maize treated both with and without glyphosate herbicides. According to the EFSA Guidance Document for the risk assessment 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 MON 89034 maize and NK603 maize in the study design at the same study sites.	The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize MON89034 x NK603. In the document “Risk assessment of plants containing genetic modification events combined by crossing” the GMO-Panel state: “Where the substantial equivalence of parental material containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one years filed trialling of events combined by crossing is acceptable where geographical localities are representative of the climatic conditions to which such crops will be exposed. Based on the outcomes of this assessment additional follow-up analysis of compositional characteristics over further growing seasons may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				trigger further assessment." Data on the pesticide treatments of the field trials has been requested and obtained. As the compositional data did not identify differences between maize MON 89034 x NK603 and the non-GM maize control of safety concern, and there are ample data on the composition of the parental lines in previous applications related to maize MON 89034 and maize NK603, respectively, the GMO Panel accepted the data supplied. The Panel notes that the parental maize was included in the field trials. Different materials of maize 89034 x NK603, maize MON 89034, maize NK603, and the non-GM control to maize 89034 x NK603 were studied for the expression of the newly expressed proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS. Expression levels were found to be comparable in the parental and stacked GM events.
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.08 Toxicology	Toxicology of the expression products is addressed by suggesting a history of safe use of the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS. However, it has to be noted that Cry1A.105 is a synthetic protein, which had never been used in commercial microbial insecticides or other transgenic plants before, while experience with Cry2Ab2 is also limited and combinatory effects of both toxins were hardly studied. A study on combinatory effects of purified Cry1A.105 and Cry2Ab2 proteins produced in recombinant Escherichia coli (MacRae et al. 2005, see application EFSA/GMO/NL/2007/39), that suggested additive activities of a toxin mixture, was only conducted with target lepidopteran species, of MON 89034 maize, while no data on other organisms were provided with application EFSA/GMO/NL/2007/38. Only a 1:1 w/w mixture of	The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize MON89034 x NK603. Both parental genetically modified events, NK603 and MON 89034, respectively, and the proteins expressed in the maize events (Cry1A.105, Cry2Ab2 and two versions of CP4 EPSPS) have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties (see

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			microbiallly produced Cry1A.105 and Cry2Ab2 was provided to the test organisms, while expression levels of Cry1A.105 and Cry2Ab2 in MON 89034 and MON 89034 x NK603 maize were shown to vary considerably (see D 3). Conclusions based on supposed but not proven analogies between other Cry toxins and Cry1A.105 and Cry2Ab2 should take this level of uncertainty into consideration. Because indications for possible adverse effects of MON 89034 maize on mammals (urinary calculi, significantly different nephrological and haematological findings) were observed in studies provided with application EFSA/GMO/NL/2007/37 for authorisation of MON 89034 maize (Kirkpatrick 2007, see application EFSA/GMO/NL/2007/37), the applicant is asked to provide a feeding study with the whole food and feed, i.e. MON 89034 x NK603 maize compared with maize with a comparable genetic background, in mammals. We suggest conducting at least a 90-day feeding study addressing, in particular, haematology and nephropathology.	previous opinions of the GMO Panel). The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional studies are needed. As Cry proteins are toxic to specific groups of insects and not to mammalian organisms, potential interaction of the Cry proteins leading to toxic effects were only studied in target insects. The parental maize events MON 89034 and NK603 were included in field trials. The harvested materials (different tissues) of these parental events, the stacked maize MON 89034 x NK603 and an appropriate non-GM control was used to study the expression of the transgenes. On request from the Panel, the applicant supplied expression data for each individual trial site and sample. The GMO Panel concluded that the expression in the stacked event was comparable to the expression in the single parental events. The Panel did not require further data to finalize its food and feed safety assessment.
Germany	Federal Agency for Nature Conservation (BfN)	D, 10 Potential changes in the interactions of the GM plant with the biotic…	Water and soil organisms may be exposed to MON 89034 x NK603 maize via the release of organic waste material, litter or sewage to the environment, which occurs during processing or through spillage. No data are provided by the applicant about the concentration of the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS in organic waste material, litter or sewage. A study addressing the aerobic degradation of Cry1A.105 and Cry2Ab2 proteins produced in recombinant Escherichia coli (Mueth et al. 2006, see application EFSA/GMO/NL/2007/38) is not considered a proper substitution for such tests. The possibility of an	The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of the GM maize MON89034 x NK603. See section 6.1.2 of the scientific opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			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.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	As stated by the applicant, the scope of the application of MON 89034 x NK603 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. • 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. •	The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion The potential extension of the monitoring beyond

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Elaboration of the methods of data analysis including the statistical methods. The monitoring should be run in regions, where MON 89034 x NK603 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.	the period of the consent is outside the remit of the EFSA GMO Panel.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.02 Case-specific GM plant monitoring	The data provided with the application are not sufficient to complete the environmental risk assessment. During transport, storage, packaging or processing incidental spillage of MON 89034 x NK603 maize can occur. Furthermore, the exposure of MON 89034 x NK603 maize and the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS to the environment during or after the production process and during animal consumption is given. Therefore, a case-specific monitoring is necessary and has to focus on pathways, how the MON 89034 x NK603 maize can get into the environment. Based on the currently available data, the case-specific monitoring plan has to comprise the following elements: • exposure of MON 89034 x NK603 maize kernels to the environment e.g. via spillage during transport, storage, packaging, processing and use, • spread, persistence and accumulation of MON 89034 x NK603 maize and the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS if spillage or loss during transport, storage, packaging, processing and use occurs, • exposure of the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS to the environment e.g. via sewage water, waste material or by-products which occur during processing. If spread, persistence and accumulation of MON 89034 x NK603 maize and the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS in the receiving environment occur e.g. via spillage, loss or release of MON 89034 x NK603 maize or of sewage water, waste material or by-products containing MON 89034 x NK603 maize, further observations of possible	The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			impacts on organisms, food chains and habitats are required.	
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 environmental risk assessment. The general surveillance plan has to focus on possible pathways how MON 89034 x NK603 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 MON 89034 x NK603 maize. The applicant is requested to provide an appropriate monitoring plan to observe the spread, persistence and accumulation of the proteins Cry1A.105, Cry2Ab2 and CP4 EPSPS in organisms and the environmental media (soil, air, water).	The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* 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 including case-specific monitoring and general surveillance have to be reported on an annual basis. All raw data have to be provided upon request. The applicant is requested to state, how the monitoring results will be published.	The EFSA 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

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				authorities. The information initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 6.1.3 of the scientific opinion
Greece	Hellenic Food Authority	D, 02 Information on the sequences actually inserted or deleted	Additional molecular analysis for the position of the inserts into the genome of the hybrid should be provided. For example, PCR amplification and sequence analysis of the MON 89034 maize insert and its 5' and 3' flanking regions as well as the NK603 maize insert and its 5' and 3' flanking regions should be conducted to confirm the stability and organization of the inserts between the GM parents and their hybrid.	Southern analysis data are considered satisfactory to confirm the stability and organisation of inserts in the hybrid.
Greece	Hellenic Food Authority	D, 07.02 Field trials | D, 07.04 Agronomic traits | D, 07.10 Nutritional assessment of GM food/feed	For the comparative assessment (agronomic, compositional and nutritional studies) as comparators were used only the non-GM nearly isogenic lines and commercial hybrids (Davis, 2006, Phillips, 2006 and Drury, 2006) 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.	The parental maize events MON 89034 and NK603 were included in field trials. The harvested materials (different tissues) of these parental events, the stacked maize MON 89034 x NK603 and an appropriate non-GM control was used to study the expression of the transgenes. On request from the Panel, the applicant supplied expression data for each individual trial site and sample. The GMO Panel concluded that the expression in the stacked event was comparable to the expression in the single parental events. The Panel did not require further data to finalize its food and feed safety assessment. See the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (EFSA,

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				2007)
Greece	Hellenic Food Authority	D, 07.08 Toxicology	Each of the introduced traits from the parental lines are inherited in MON 89034 × NK603 maize, which results in the expression of the Cry1A.105, Cry2Ab2 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 4 introduced proteins in the hybrid and not with each one transformation event (GM parents) separately.	Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional animal studies are needed.
Italy	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	General comments	The followings are the comments sent by C.A. to the European Commission on the monitoring carried out by Monsanto on the individual event NK603. The report presented by the notifier cannot be considered completely satisfactory because of the following reasons: 1. The notifier hasn't provided any data on the quantity of NK603 seeds imported in Europe, asserting that he isn't the only operator directly involved in NK603 seeds trading. However, being the notifier responsible for the general surveillance on potential effects related to the placing on the market of NK603 maize, he should be informed on the quantity and the places where the NK603 was sold. 2. The notifier arranged "early warning" system in Spain through a unique and direct contact which only covers the Iberian market. For the other Member States a system that collects all information provided by category delegates on an annual basis has been set up. This mechanism is structured on a voluntary basis, not standardized in the	Application EFSA/GMO/NL/2007/38 concerns the GM maize MON89034 x NK603. Dossiers for placing the GM maize NK603 on the market (notification C/ES/00/01 under Directive 2001/18/EC and a notification under Article 4 of Novel Food Regulation (EC) No 258/97) for import and processing of maize NK603 for food and feed uses were assessed by the GMO Panel. The EFSA GMO Panel was of the opinion that maize NK603 is as safe as conventional maize. Therefore their placing on the market for food and feed uses as well as processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment (EFSA, 2003a, b). Maize NK603 has received an opinion in favour of

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			data format (except for Spanish and Portuguese markets). This system is to be considered not adequate. 3. Among the networks selected by the notifier for the implementation of the monitoring plan, only the industry associations, of manufacturers and food and feed supply chain are present. No other surveillance networks, that are listed in table 1 of the monitoring plan of the notification,, are considered. Particularly, considering the destination of the imported maize grains, as feed, the contacts with the national monitoring networks for veterinary products could provide important information on potential damages to the health of livestock fed with maize NK603. 4. Moreover, the notifier should demonstrate that the surveillance networks indicated on the monitoring plan of the notification do collect and do send information which are useful to the general surveillance of the product as required by Commission Decision 2004/643/CE (art.4.4). The notifier should also indicate clearly the methodology used,; 5. The notifier is required to demonstrate that the statement included at pag.11 (paragraph 8, last section) of the report presented by the notifier itself is the result of an epidemiological analysis and is not derived from simple conjectures or hypothesis. Finally, as asserted by the notifier, maize NK603 can be considered as safe as traditional maize (the commercialisation within the EC is authorized) but scientific uncertainties about the indirect, cumulative and delayed potential effects still remain.Hence, the notifier is requested to integrate the monitoring plan with the relevant points above and to implement the general surveillance plan according to the requirements of Commission Decision 2004/643/CE with the greatest care.	its authorisation and was authorised under Directive 2001/18/EC by Commission Decision 2004/643/EC. The use of food and food ingredients from NK603 maize was authorised under Regulation (EC) No 258/97 by Commission Decision 2005/448/EC.
Italy	Ministero dell'Ambiente e della Tutela	D, 08 Post-market monitoring of	The Post market monitoring plan submitted by the applicant, does not provide the necessary and detailed information and data requested in the EFSA"New chapter	Section D.08 deals with potential post-market monitoring of GM food and/or feed, and not to post-market environmental monitoring. No

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
	del Territorio e del Mare	GM food/feed	11.4: General Surveillance of unanticipated adverse effects of the GM Plant", even if the applicant reminds to the Guide Lines of EFSA itself. In particular: -the applicant does not identify the Existing Monitoring Programmes that would be used to perform the General Surveillance; -the time schedule of monitoring activities is not indicated; -there isn't a description of any "early warning system" in case of the identification of an unanticipated effect; -the applicant doesn't give any information related to which stakeholder (associations, seed ……) will be contacted and how; the applicant doesn't give any information on the methods that will be used to collect data and information. Moreover, the applicant affirms that: "As the import aspect covered by both Mon89034 and NK603 applications is identical, (to the Notification C/ES/00/01), the applicant believes that the GS plan endorsed by EFSA for NK603 can also serve as a model for Mon89034". The ANC gave its comments on the annual report submitted by applicant on event NK603 and reported in general comments. Therefore, after considering the comments the applicant must integrate and develop the monitoring plan as required.	potential food and feed safety issue was identified that requires to be addressed by post-market monitoring. The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion See response to the hereabove comment under 'General comments' from Italy

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) 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				
Norway	Norwegian Scientific Committee for Food Safety	D, 07.09 Allergenicity	7.9.2 Assessment of allergenicity of the whole GM plant or crop 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 MON 89034 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 Cry1A.105 and Cry2Ab2 protein in food and feed from maize MON 89034 cannot be excluded. Thus, the Panel's view is that as the adjuvant effect of mCry3Bb1 with reasonable certainty cannot be excluded, the applicant in relation to a possible adjuvant effect of Cry1A.105 and Cry2Ab2 must comment upon the mouse studies showing humoral antibody response of Cry1A proteins. Further, although the Cry1A.105 and Cry2Ab2 protein is rapidly degraded in gastric fluid after oral uptake, there is also the possibility that the protein can enter the respiratory tract after exposure to e.g. mill dust. Finally, rapid degradation is no absolute guarantee against allergenicity or adjuvanticity. 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	Immunogenicity and adjuvanticity of Cry proteins have been described in several publications. After intraperitoneal or intragastric administration of Cry1Ac to mice at relatively high dosage, IgG, IgM and mucosal IgA response were induced, but no IgE response was observed (Vazquez-Padron et al., 1999a,b; 2000). This demonstrates that the immunogenicity of Cry1Ac is not associated with an allergenic potential. This is further supported by recent bioinformatic studies carried out by the Swedish National Food Administration using a newly developed methodology (Soeria-Atmadja et al., 2004; Bjorklund et al., 2005) showing the absence of sequence homology between Cry1Ac and known allergens (unpublished results). No other adverse effects were observed when using the oral route of administration. On the other hand, Cry1Ab has been shown to act as an adjuvant e.g. it enhances the mucosal and/or the systemic antibody response to a protein which is co-administered with the Cry protein after high dosage intragastric or intranasal administration (Vazquez et al., 1999a,b; Moreno-Fierros et al., 2003). The authors suggested that Cry1Ac could thus be used as a safe adjuvant in oral vaccination.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) 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				
			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. Vazquez-Padron RI. Moreno-Fierros L. Neri-Bazan L. Martinez-Gil AF. de-la-Riva GA. Lopez-Revilla R., 2000b. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz J Med Biol Res., 33: 147-55.	
Norway	Directorate for nature management	A. General information	The application does not include cultivation in Europe, but according to the Norwegian Gene Technology Act possible contributions to a sustainable development, 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. Thus, we would, in order to facilitate an approval in Norway, like the applicant to elaborate on the effects of the maize hybrid 89034xNK603 on these subjects.	Outside the remit of the GMO Panel
Norway	Directorate for nature management	A. General information	A change in agricultural practice may have environmental impact on non-target species and result in changes in growers and consumers exposure to pesticides leading to altered levels of health risk. We would like the applicant to specify what changes in agricultural practice, e.g. use of pesticides, Mon89034xNK603 is expected to lead to in the areas where it is expected to be grown.	The scope of the application is for import, processing as well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	We would like to ask the applicant to provide a list of persons/organisations that applicant regards as key stakeholders and in that respect will be asked to take part in the general surveillance. Consent for marketing of Mon89034xNK603 should not be given until agreements with the chosen persons/organisations have been made in order for the Member States to evaluate the choice of participants.	The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	We do appreciate that the consent holder is responsible for the reporting of results of the surveillance. On the other hand, we do feel that the general description of responsibilities for the different steps in the monitoring plan is not adequately described. According to Council decision 2002/811/EC, the responsibility for each step in the monitoring plan should be clearly assigned by the	The EFSA 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 initially supplied and

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			applicant. Surveillance is a key prerequisite for the approval of a GMO, and so it is essential that the responsibilities of the contributing parties in the surveillance are defined	supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion
Spain	MINISTRY OF THE ENVIRONMENT	D, 02 Information on the sequences actually inserted or deleted | D, 07 Information on any toxic, allergenic or other harmful effects on human or… |	SPANISH COMMENTS EFSA/GMO/NL/2007/38: MON 89034 x NK603 MAIZE Comments of the National Commission on Biosafety of Spain D.02. Information on the sequences actually inserted or deleted The molecular characterisation is complete and the results can be considered satisfactory, although we suggest that generally it could be desirable to have Southern analysis of better quality for example in the use of the specifics probes for monitoring each gene insertion. The notifier should submit the chromosomic location of the inserts in the hybrid that it could be relevant for potential protein interaccion. D.7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food and feed D.07.08 - Toxicology. The notifier should submit	Chromosomal locations of the inserts are not relevant to the safety of this product. Protein expression levels in the single events are comparable to the hybrid and do not raise any safety concern. There are no indications from comparative agronomic performance and compositional analyses of any unintended effect caused by the combination of the single events in the hybrid. Both parental genetically modified events, NK603 and MON 89034, respectively, have previously been assessed for potential toxicity by the GMO-Panel and found to be as safe as

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
		D, 07.08 Toxicology | D, 07.09 Allergenicity	acute and subchronic studies with all proteins (Cry1A.105 and Cry2Ab2 and CP4 EPSPS) together since we consider that studies made with proteins separately are not enough for carrying out the overall risk assessment of these GMO products. We believe that the current EFSA Guidelines document for the hybrids is not enough detailed in this sense. D.07.09-Allergenicity Sequence homology searches: Comparison of 8 amino acids sequences are used, what facilitates the existence of false negatives. The approach FAO-OMS (2001) (comparison of 6 amino acids), is not applied and in our view that offers bigger sanitary security. Therefore, similarities with well-known allergens are still possible (there are examples of different epitopes of highly allergenic proteins of 6 amino acids) but they have not been detected due to this to proceed. Respect to Digestion studies: The experiments with proteins (Cry1A.105 y Cry2Ab2) degradability need to be more expanded, in particular regarding digestion with different pHs and including the characterization of degradation products, essentially their size and sequence of the long polypeptide sequence proteins. In none of the two tests it indicates that it has been used a digestible protein (for example, bovine seroalbumine) neither a non-digestible protein (for example, allergens of the peanut), to contrast the effectiveness of the test. We believe that in the case of Cry1A.105 digestion test requires an in-depth study, concretely on the 4.5 KDa polypeptide fragment obtained after twenty minutes in the digestion assay, which is big enough to contain inmunoreactive sequences.	conventional maize varieties. The panel has identified no additional hazard due to interaction of the transgenes expressed in the two parental maize lines. The panel is of the view that no additional studies are needed. As is evident both from the Codex Alimentarius document on the Conduct of Food Safety Assessment of Foods derived from r-DNA Plants and the Guidance document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed, bioinformatics searches for identical stretches of 6 contiguous amino-acids of the test proteins to stretches of the same size in known allergenic proteins is not advocated. This is partly due to the very high likelihood of non-allergenic proteins then being identified as allergens. This issue will be further elaborated on in a report from an working group under the GMO Panel dealing with allergenicity risk assessment. Although the present application contains no digestibility studies, the MS addresses issues related to such studies. The Panel assumes that the MS is referring to data in the application for the parental event MON 89034. The issue related to the degradation fragment of Cry1A.105 observed already in the application to introduce maize MON 89034 on the EC market, was addressed already when assessing this parental event; see earlier opinion from the GMO Panel. However, it can be mentioned that the allergenicity report soon to be published also address issues related to pepsin digestion studies.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) 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				
The Netherlands	Ministry of of Agriculture, Nature and Food Quality and the Ministry of Health	D, 07.01 Comparative assessment	In various comparative studies (compositional analysis, phenotypic and agronomic characteristics, 42-day broiler study), MON 89034 x NK603 maize has been compared with a control maize stated to be of similar genetic background as MON 89034 maize (LH198 x LH172 or H1325023). It is not clear whether these control maizes are also representative for MON 89034 x NK603 maize. The applicant should therefore be asked to provide more data on the pedigree of MON 89034 x NK603 maize and its controls as used in the various trials reported in the dossier. In addition, in none of the trials the GM parental maizes were included as controls. For a proper evaluation of the safety of MON 89034 × NK603 maize, data on these controls should be provided.	Data on the relationship between the control maize and maize MON 89034 x NK603, including a pedigree, was requested and obtained.
The Netherlands	Ministry of of Agriculture, Nature and Food Quality and the Ministry of Health	D, 07.08 Toxicology	As previously noted for the import of MON89034: The SDS-PAGE and Western blot results show a clear additional proteolytic fragment at ~90 kDa in E. coli produced Cry1A.105, which is barely visible in MON 89034 produced Cry1A.105. In addition, the SDS-PAGE and western blot results of Cry2Ab2 show an additional proteolytic fragment at ~ 50 kDa in MON 89034 produced protein, which is not present in E. coli produced protein, and a larger (~ 130 kDa) (not further specified) fragment in E. coli produced Cry2Ab2, which is not observed in the lanes of MON 89034 produced Cry2Ab2. Whereas the applicant has shown that the smaller fragments are degradation fragments, there is no explanation for the larger fragment in E. coli produced Cry2Ab2. Although it is not likely that this larger fragment	The GMO Panel addressed safety issues related to the single events when these were assessed – see previous opinions of the GMO Panel.

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) Comments and opinions submitted by Member States during the three-month consultation period				ANNEX G
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			has toxicologic effects, we would like to have an explanation for its origin and for the difference in patterns of the fragments (including the degradation products) between E. coli and MON 89034 produced Cry proteins before conclusions can be drawn on the physicochemical and functional equivalence.	
The Netherlands	Ministry of of Agriculture, Nature and Food Quality and the Ministry of Health	D, 07.08 Toxicology | D, 07.10 Nutritional assessment of GM food/feed	In the 42-day broiler study information on sex differences is lacking. This should be provided.	The GMO Panel has asked the applicant to supply a statistical evaluation of the nutritional broiler chicken feeding study for each sex separately. The requested information was obtained.
The Netherlands	Ministry of of Agriculture, Nature and Food Quality and the Ministry of Health	D, 07.10 Nutritional assessment of GM food/feed	In the 42-day broiler study with MON 89034 x NK603 maize, the starter diet contains approximately 58% w/w maize (as compared to approximately 55% in most broiler studies), making it not much different from the grower/finisher diet (59.5%). Why is the amount of maize in the starter diet relatively high?	The GMO panel agrees that 58% maize in the starter diet is relatively high and the increase to 59,5% in grower/finisher diet is only small. But the requirements of all other essential nutrients are met in the starter, grower and finisher diets. The maize portions in the diets are not a direct matter of safety concern, because the levels of maize in all diets are high enough for safety assessment of GM-maize. It is recommended in most guidelines to include the maximum levels of feeds, which should be tested.
The Netherlands	Ministry of Housing, Spatial Planning and the Environment	C, 01 Description of the methods used for the genetic modification	As previously noted for the import of MON 89034: The A. tumefaciens strain ABI, which has been used to genetically modify MON89034, contained a helper plasmid without any T-DNA regions. However, it is unclear whether other plasmids, that may contain additional T-DNA regions, are present within ABI. The applicant should provide information about the presence or absence of other plasmids and additional T-DNA regions in A. tumefaciens strain ABI.	The ABI *Agrobacterium* strain is well characterised. Under good laboratory practices, contamination of the strain with other plasmids is not a reasonable assumption.
The Netherlands	Ministry of Housing, Spatial	D, 02 Information on the	As previously noted for the import of MON89034: Bioinformatic analyses of the putative polypeptides in the junctions between the insert and the maize genomic DNA	Bioinformatic analyses indicate that should any of the putative ORFs be translated, none of the potential peptides would show homology with

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
	Planning and the Environment	sequences actually inserted or deleted	did not indicate any structural similarity to allergens or toxins. However, the large T-DNA border sequences at the 5' and the 3' end of the insert were not fully analyzed. Putative polypeptides present in the complete border sequences should be assessed for similarity to allergens or toxins.	known allergens, toxins or other biologically active peptides. The applicant has provided an up to date (2007) bioinformatic analysis of the region spanning the T-DNA and genomic DNA (5' 932 bp, 3' 2050 bp), see MSL0020938. For the safety assessment, only newly created ORFs are considered to be relevant, also taking into account the extensive knowledge on the T-DNA borders.
The Netherlands	Ministry of Housing, Spatial Planning and the Environment	D, 12.03 General Surveillance of the impact of the GM plant	General surveillance will be performed by key stakeholders and key networks of stakeholders. The permit holder will request key stakeholders and networks to participate and asks them to be informed if any unanticipated adverse effects occur. However, it is unclear how these effects are monitored if key stakeholders and networks do not assist. The permit holder should ascertain that information on adverse effects is obtained even if key stakeholders and networks do not participate.	The EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant*

Application EFSA-GMO-NL-2007-38 (MON89034 x NK603 Maize) **Comments and opinions submitted by Member States during the three-month consultation period**				**ANNEX G**
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				after the application has been accepted (…). See section 6.1.3 of the scientific opinion
The Netherlands	Ministry of Housing, Spatial Planning and the Environment	D, 12.06 Reporting the results of monitoring	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 EFSA 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 initially supplied and supplemented in the course of the risk assessment by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.1.3 of the scientific opinion

The secretariat have to give input on page 7, 31 and 34, and Gerhard on page 36. The secretariat should also check all instances when references is made to the opinion, as the numbering of the opinion has been changed. Finally, the internet translation of the French comment and my suggested answer should be checked.