Application EFSA-GMO-UK-2005-11 (Maize MIR604) **ANNEX G** **Comments and opinions submitted by Member States during the three-month consultation period**				
Country	**Organisation**	**Reference**	**Comments**	**EFSA GMO Panel responses**
Comments from National Competent Authorities under Directive 2001/18/EC				
Denmark	Danish Forest and Nature Agency	General comments	A quantitative detection method should be validated before placing on the market.	Outside the remit of the EFSA GMO Panel
Finland	Board for Gene Technology	General comments	The comment of the Board for Gene Technology: Before marketing in certain areas the consent holder should ensure that a general monitoring plan which takes into account the specific conditions of that area and the extent of the release is put into place.	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 supplied by the applicant is in line with this guidance. The applicant provided a '*Monitoring plan for the import and use of GM maize event MIR604 in the EU*' according to Annex VII of Directive 2001/18/EC, including a methodology for the general surveillance of viable maize MIR604. See section 6.1.2. of the scientific opinion In addition, 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*

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				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 (…).
France	Ministère de l'agriculture et de la pêche/DGAL	General comments	L'évaluation des risques pour l'environnement conduite par la Commission du génie biomoléculaire (avis du 16 novembre 2005) n'a pas mis en évidence de risques pour l'environnement liés à la mise sur le marché du maïs MIR604, telle que décrite dans le dossier EFSA/GMO/UK/2005/11	The EFSA GMO Panel agrees with the French Commission du génie biomoléculaire.
Germany	Federal Office of Consumer Protection and Food Safety	General comments	1) The German CA is of the opinion that further information is required to conclude the risk assessment of dossier EFSA/GMO/UK/2005/11. Diverging and additional comments from cooperating authorities (Federal Agency for Nature Conservation) are included for information and indicated. 2) Comments by the Federal Agency for Nature Conservation: The monitoring plan provided by the applicant does not meet the requirements of Annex VII of Directive 2001/18/EC. The monitoring plan should therefore be amended	1) See section of the scientific opinion: *Overall Conclusions and Recommendations* 2) 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 supplied by the applicant is in line with this guidance. The applicant provided a '*Monitoring plan for the import and use of GM maize event MIR604 in the EU'* according to Annex VII of Directive 2001/18/EC, including a methodology for the general surveillance of viable maize MIR604. See section 6.1.2. of the scientific opinion

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				In addition, 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 (…).*
Norway	Directorate for Nature Management	A. General information	1) The notification lacks sufficiently detailed information on possible contributions of MIR604 to a sustainable development, benefits to the society and other ethical considerations regarding the use of maize line MIR604. These aspects will be addressed in the evaluation of the notification in Norway under the Norwegian Gene Technology Act. 2) Amongst others we request information on expected areas of cultivation and the specific changes in cultivation practices that use of MIR604 has lead to.	1) Outside the remit of the EFSA GMO Panel 2) The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604.
Malta	Malta Environment and Planning Authority	A, 04 Scope of the application as defined in Annex II	The notification concerns a request for maize bearing the event plus any inbred or hybrids. The Maltese Competent Authority is of the opinion that separate notifications are required for the different plant events.	This scientific opinion concerns only a single event which is MIR604 analyzed in different genetic backgrounds. The scope of the application includes all feed and food products containing, consisting or produced

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				from the genetically modified maize Event MIR604 including products from inbreds and hybrids obtained by crosses with conventionally breed maize.
Germany	Federal Office of Consumer Protection and Food Safety	A, 07 Where appropriate, the conditions for placing on the market the food(s) or…	The accompanying shipping documents should indicate that maize seed "MIR604" has not been approved for cultivation. Appropriate measures have to be taken during transport, storage and processing to avoid unintended release to the environment.	This comment is related to management measures and therefore falls outside the remit of the EFSA GMO Panel.
Malta	Malta Environment and Planning Authority	C, 01 Description of the methods used for the genetic modification	Further evidence to support the robustness of the PCR based detection method is required as requested by the Joint Research Centre (JRC). · The points raised by the JRC regarding the limit of quantitiation of the PCR test which needs to be established through a separate test and the reservations concerning how sensitive the test is at lower levels need to be clarified.	Outside the remit of the EFSA GMO Panel
Italy	Ministero Ambiente e Territorio	D. Information relating to the GM plant | D, 01 Description of the trait(s) and characteristics which have been introduced…	In APPENDIX I CBI "Molecular Characterization of Event MIR604 Maize (Corn) Expressing a Modified Cry3A Bacillus thuringiensis Protein" are reported data concerning the Southern analysis of Event MIR604 . Maize genomic DNA (7.5 µg) was digested only with KpnI restriction enzyme. However, we retain that these data are insufficient, indeed genomic DNA digestion should be performed also with others restriction enzymes. Moreover, mcry3A (Figure 4) and pmi (Figure 6), MTL probes the KpnI digest resulted in a single hybridization band of the same size.	The EFSA GMO Panel is satisfied that the information provided by the Southern analysis in combination with sequencing and inheritance studies is sufficient to indicate insertion of a single T-DNA copy. Hybridisation patterns using *Kpn*I digests and *mCry3A*/*pmi* probes indicate different band sizes (about 5.2 and 5.8 kb). See section 3.1.4 of the scientific opinion

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Austria	Ministry of Health and Women	D, 01 Description of the trait(s) and characteristics which have been introduced…	1) The comparison of the plant inserted T-DNA sequence with the plasmid T-DNA showed that the inserted T-DNA contains modifications within the coding sequence of the PMI marker gene giving rise to two amino acid changes. The applicant refers to an internal report [Hill, K. (2004) Characterization of Phosphomannose Isomerase (PMI) Produced in Maize (Corn) Plants Derived from Event MIR604 and Comparison to PMI as Contained in Test Substance PMI-0198. Unpublished Syngenta data volume dated October 18, 2004.] which addresses the biochemical ramifications of the amino acid changes. However, this report is not included in the application. Any conformational changes or other possible consequences for the secondary or tertiary structure of the protein resulting from these amino acid changes should be evaluated and subject to a thorough risk assessment. 2) The phenotypic stability of the insert was demonstrated by analysis of protein levels over four backcross generations in leaves only but not in any other plant tissues (Appendix III). Given the relevance of this transgenic hybrid for root pests, evaluation of stability of protein expression in root tissues should be included. As this maize is intended to be used for food and feed purposes stability of the introduced traits including expression of the corresponding proteins in kernels should be demonstrated. Instable expression of inserted proteins may have an effect on the quality and the safety of the product imported into the EU, e.g. due to consequences for toxicology or allergenicity or via possible secondary effects.	1) Two of the base pair changes result in amino acid substitutions in the phosphomannose isomerase (PMI) protein. The proteins showed in Western analysis similar size and immunoreactivity. The enzymatic activity was comparable and no evidence of glycosylation was detected. See section 3.1.2 of the scientific opinion 2) Level of mCry3A protein in kernels has been assessed in two hybrids and one inbred line background over three generations. See section 3.1.3 of the scientific opinion The root expression levels of the transgenic proteins, in particular mCry3A and PMI, might have been of interest if the plant were to be grown within the EU. The levels of mCry3A and PMI in root tissue of MIR604 have been provided for one season. Data on the agronomic performance of MIR604 indicate that it has less corn rootworm damage than controls during multiple seasons, indirectly indicating the effectiveness of expressed mCry3A proteins. Given that the application pertains to import, processing, food and feed use of *kernels*, and that the applicant has investigated the stability in leaf tissue over four generations, there is no particular rationale for requesting information on root tissue expression.

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Germany	Federal Office of Consumer Protection and Food Safety	D, 03 Information on the expression of the insert	Comments by the Federal Agency for Nature Conservation: With regard to a final assessment further information is required. The expression of the insert was tested in (Illinois) USA (one season, one location, two maize hybrids, one maize inbred, four growth stages). Results of the trial are presented in Appendix III (Joseph & Hill, 2003) of the application, respectively. The analysis for the range of the expression of mCry3A and PMI relies solely on one field trial in one location. Since the expression can be affected by climatic conditions, soil fertility, agricultural practice or unknown gene-environment interactions, the data presented give only a crude estimate of the range and can not be regarded as sufficient for a market release.	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604. For further details on the expression of the insert, please see section 3.1.3 of the scientific opinion.
Austria	Ministry of Health and Women	D, 07.03 Selection of compounds for analysis	The results of the compositional equivalence analysis show significant differences for several parameters between the GM maize compared to the non-GM maize hybrids (i.e. Calcium levels in grain were 4-10% higher in one growing season in GM corn; some vitamins were 9-18% lower in GM corn in one season, oleic acid was different in both seasons, etc., Appendix IV). Differences of this extent should not be regarded as biologically not significant. Statistically significant differences in composition of GM and non-GM maize hybrids grown and harvested under the same conditions should trigger further investigations as to the relationship between the difference and the genetic modification process. Additionally, plant cell wall components for the vegetative parts were not examined as required by EFSA (2004) for plants	The current application pertains to import, processing, food and feed use of kernels, while the compositional data on forage have been taken into account as well. As regards the plant cell wall components of vegetative parts, fibre has been measured in forage derived from MIR604. The differences in kernels mentioned by the member state are also considered in the EFSA GMO Panel's opinion. The only difference that was consistently observed in both seasons in one hybrid pair was that of oleic acid. It is known, however, that fatty acid composition of maize is variable, such as for oleic and linoleic acid (Dunlap et al., 1995). In addition, differences in oleic acid levels *per se* do not pose a safety hazard. As regards the B vitamins that were decreased in the transgenic maize during one

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			used for feed purposes.	year, these values were still within the background range. In addition, these differences were not consistent as they occurred in one year only. It should also be noted that the standard methods that had been used for testing B vitamins relied upon microbiological assays (this is mentioned in the appendices to the report on compositional analysis). With regard to calcium levels changes, additionally provided data show that these differences were not consistent across location and that the GM line did not show any consistent higher or lower content of calcium across location. Thus the differences observed are considered by the EFSA GMO Panel to be minor and neither pose any safety hazards nor trigger any further investigation.
Malta	Malta Environment and Planning Authority	D, 07.04 Agronomic traits	The agronomic tests relating to the testing of plant characteristics were carried out on 'Mir-derived hybrids' (see section 2). Could the notifier specify if these varied from Mir604? And if so how?	MIR604 refers to the initial transformation event including all its progeny. The initial transformant was a hybrid of two maize inbred lines. It was backcrossed with one of its parental inbreed lines (NP2500) and selfed several times to provide the germplasm of NP2500 as the genetic background. Furthermore MIR604 in the germplasm of NP2500 was crossed with non transgenic maize lines to produce F1 hybrids (see section 3.2.1). Genetic and phenotypic stability of the event MIR604 has been demonstrated. See section 3.1.4 of the scientific opinion

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Malta	Malta Environment and Planning Authority	D, 07.04 Agronomic traits	The agronomic tests relating to determination of the levels of plant growth characteristics (Steiner 2004) are cross referenced in the section 'part 1, technical dossier- part 1 – confidential information'. However the reference only appears as a front page and the full details of the tests and results could not be viewed. Has the notifier presented this information or does it only exist as a quoted reference in the above mentioned section? · Likewise the analytical tests on levels of fatty acids, moisture etc which were carried out to show that the transgenic plant is not significantly different from the conventional plant were not located. Has Kramer 2004 been presented by the notifier or does it only exist as a quoted reference in Appendix IV? · It was not clear whether parameters associated with increased risk of weediness such as dormancy, plant vigour, pollen production and dissemination have been assessed and whether these are the same as in the parent plant.	The agronomic data (Steiner 2004) have been included as confidential appendix part I – CBI-4. As mentioned in the EFSA GMO Panel's opinion, the parameters tested included corn rootworm damage, pathogen infestation, yield and other physiological characteristics, including dropped ears, plants emerged, ear height, grain moisture, plant density, plant intactness, heat units and days until both silking and pollen shed, root lodging, plant height, stalk and root quality, stalk lodging, and kernel test weight. The EFSA GMO Panel considered that besides the expected differences in agronomic performance that are linked with the introduced insect-resistance trait of maize MIR604, the performance of this maize can be considered as being equivalent to that of conventional maize. The full text of Appendix IV (Kramer 2004) has been provided in the non-confidential part of the appendices to the technical dossier.
Austria	Ministry of Health and Women	D, 07.08 Toxicology	1) For the safety evaluation of the newly expressed proteins the applicant provides an acute oral toxicity study (14 days) with the microbially produced mCry3A protein and the PMI marker protein (Appendices VI and VIII) as well as a homology search to known toxins, evaluation of influence of temperature and a digestion analysis of the proteins in simulated gastric fluids which should substitute the 28 days oral toxicity study. Generally, little significance can be attributed to toxicological tests with isolated gene products, already mentioned by many authors [Spök A., Hofer H., Lehner P., Valenta	1) The EFSA GMO Panel agrees that the acute toxicity studies are of limited value for the assessment of maize MIR604. Besides these acute toxicity tests, however, the total package of safety testing that has been performed on MIR604 and transgenic proteins included i) subchronic rodent feeding study with the whole product; ii) *in vitro* digestibility of transgenic proteins; iii) bioinformatic-supported comparisons of the primary structures of the transgenic proteins with allergens and toxic proteins, as well as a comparison of the spatial

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			R., Stirn S. Gaugitsch H. (2005). Risk Assessment of GMO Products in the European Union. Umweltbundesamt Wien, Band 253. Millstone E. (1999), Beyond substantial equivalence. Nature 401 (6753): 525-526, Walker R. (2000). Joint FAO/WHO Expert consultation on foods derived from Biotechnology. 29 May-2 June 2000. Geneva.] due to the fact that pleiotropic effects in the plant as well as differences in protein quality remain unconsidered. There is scientific evidence that these parameters studied do not necessarily prove the toxicological or allergological safety of proteins. Moreover, in the toxicological studies (acute oral toxicity study of mCry3A and PMI protein in mice; Appendices VI and VIII) OECD testing guidelines are not followed as recommended by EFSA [EFSA (2004). Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99, 1-94] . 2) However, if a microbial substitute is used as a test substance for the assessment of toxicological safety of a genetically modified plant then the structural, biochemical and functional equivalence of this microbial substitute with the newly expressed protein in the GM plant must be demonstrated. The methods used for the demonstration of equivalence of PMI or mCry3A proteins indicate that the microbial substitute proteins differ from the plant-derived protein. The evaluation of the substantial equivalence of the microbial substitute for the PMI protein and the plant-derived protein shows that the	structures of PMI and an allergenic protein from peanut (Ara h 1), both being member of the cupin superfamily; iv) analysis of functional characteristics, including substrate specificity and pH-activity relationship, of PMI; and v) equivalence of the microbiologically produced analogues of the transgenic proteins to the plant-expressed ones. This package is considered by the EFSA GMO Panel as being sufficient. 2) The EFSA GMO Panel has received additional information on the identity of the protein bands observed during Western blotting. For example, mass spectrometry has been carried out on transgenic mCry3A proteins and the lower MW band (55,000 Da) has thus been identified as a C-terminal fragment of mCry3A. In addition, an additional acute toxicity study has been provided with a microbial analogue of PMI expressed in plants. Based on the data provided by the applicant, it was noted that the microbiologically produced analogues of mCry3A and PMI contain

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			plant derived protein has a slightly lower molecular weight than the PMI protein produced by E. coli (Appendix II). Additionally, the enzymatic activities of the leaf extracts show a large variation in the specific activity of the enzyme although only three leaves were tested while the activity of the microbially produced enzyme was much lower. 3) For a thorough protein characterisation and comparative analysis a fingerprinting method based on mass spectrometry or similar methods are recommended. The evaluation of the equivalence of the microbial substitute and the plant-derived mCry3A protein showed an additional band of lower molecular weight (~55,000 Da) which was only present in the plant-derived protein (sample IAPMIR604-0103). The applicant suggests that this protein is most likely derived from in planta degradation of the mCry3A protein. However, this assumption is not verified by the applicant. In case an additional protein is produced in the genetically modified plant this protein should be subject to a thorough risk assessment procedure. 3) Additionally, the insecticidal activity against one target pest species differed considerably between the mCry3A from E. coli and the protein isolated from MIR604 maize. The LC50 value of the microbially produced toxin was on average double (0.43 µg mCry3A/ml diet) compared to the LC50 value of the toxin expressed in MIR604 maize plants (0.20 µg	N-terminal extensions. However, these recombinant analogues have retained their functional activities as evidenced by insect bioassays for the two separate forms of mCry3A and enzymatic assays for PMI. The EFSA GMO Panel recognizes that enzymes and insecticidal proteins expressed in plant materials like leaves are naturally characterized by variation of their biological activity, (e.g. influences of matrix components). Taking this variation into account and the fact that the values measured by the applicant are well within the same order of magnitude, plus the other data including mass spectrometry, immunoblotting, and glycosylation assays, the EFSA GMO Panel therefore considers that the plant-expressed transgenic proteins and microbiologically produced analogues are similar. 3) About the extra-band (55 Da), a mass spectrometry confirms that extra-bands with a lower molecular weight were mCry3A breakdown product.

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			mCry3A/ml diet). Therefore, it cannot be concluded that the microbially produced mCry3A protein is equivalent with the plant-derived protein.	
Austria	Ministry of Health and Women	D, 07.08 Toxicology	Second part: 1) The evaluation of the substantial equivalence of the microbial substitute for the PMI protein and the plant-derived protein shows that the plant derived protein has a slightly lower molecular weight than the PMI protein produced by E. coli (Appendix II). Additionally, the enzymatic activities of the leaf extracts show a large variation in the specific activity of the enzyme although only three leaves were tested while the activity of the microbially produced enzyme was much lower. For a thorough protein characterisation and comparative analysis a fingerprinting method based on mass spectrometry or similar methods are recommended. 2) The evaluation of the equivalence of the microbial substitute and the plant-derived mCry3A protein showed an additional band of lower molecular weight (~55,000 Da) which was only present in the plant-derived protein (sample IAPMIR604-0103). The applicant suggests that this protein is most likely derived from in planta degradation of the mCry3A protein. However, this assumption is not verified by the applicant. In case an additional protein is produced in the genetically modified plant this protein should be subject to a thorough risk assessment procedure. 3) Additionally, the insecticidal activity against one target pest species differed considerably between the mCry3A from E. coli and the protein isolated from MIR604 maize.	1-4) The EFSA GMO Panel has received additional information on the identity of the protein bands observed during Western blotting. For example, mass spectrometry has been carried out on transgenic mCry3A proteins and the lower MW band has thus been identified as a C-terminal fragment of mCry3A. In addition, an acute toxicity study has been provided with a microbial analogue of PMI expressed in plants. Based on the data provided by the applicant, it was noted that the microbiologically produced analogues of mCry3A and PMI contain N-terminal extensions. However, these recombinant analogues have retained their functional activities as evidenced by insect bioassays for the two separate forms of mCry3A and enzymatic assays for PMI. The EFSA GMO Panel recognizes that enzymes and insecticidal proteins expressed in plant materials like leaves are naturally characterized by variation of their biological activity, (e.g., influences of matrix components). Taking this variation into account and the fact that the values measured by the applicant are well within the same order of magnitude, plus the other data including mass spectrometry, immunoblotting, and glycosylation assays, the EFSA GMO Panel therefore considers that the plant-expressed transgenic proteins and

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			The LC50 value of the microbially produced toxin was on average double (0.43 µg mCry3A/ml diet) compared to the LC50 value of the toxin expressed in MIR604 maize plants (0.20 µg mCry3A/ml diet). 4) Therefore, it cannot be concluded that the microbially produced mCry3A protein is equivalent with the plant-derived protein. 5) The applicant provides a rat and a poultry feeding study with the whole MIR604 maize plant for 90 and 49 days in order to demonstrate toxicological safety as well as nutritional equivalence of whole GM maize plant (Appendices IX and X). These studies measured mainly commercial parameters (body weight, weight gain, feed conversion, survival, carcass and part yield) representing feed conversion studies and not toxicological safety studies. Toxicological safety studies must take into account toxicological endpoints. Moreover, several observations in the 90 day feeding study with rodents (see Appendix IX) should trigger further investigations. The statistically significant lower food consumption of male rats in both GM corn inclusion groups indicates that male rats did not feed as much on GM corn as on non-GM corn over the whole feeding period. A difference in bodyweight of 9% at the end of the experimental period cannot be considered as "small". Furthermore no interpretation or statistical evaluation of microscopic findings of observed lesions in several organs was carried out. The effect of processing on the presence of proteins in different fractions was only evaluated for the mCry3A protein (Appendix V) but not for the PMI protein. Taking into account	microbiologically produced analogues are similar. 5) The EFSA GMO Panel acknowledges the fact that poultry feeding studies commonly provide data on the nutritional characteristics. It thus addresses this issue under the heading of "nutritional assessment of the GM food/feed" in its scientific opinion (see Section 5.1.5). The EFSA GMO Panel has taken notice of the decreased consumption in males on 41.5% GM diet lower in weeks 1, 2, 3, 5, and 6, and in females on both GM diets in week 2. The EFSA GMO Panel also mentions the observed decrease in bodyweights in males fed diets containing 10% MIR604 maize in its scientific opinion. Differences in bodyweights were only transiently observed in other groups fed diets containing 10% (female) and 41.5% (male, female) GM maize. There is therefore neither an apparent consistency nor a dose-response relationship in these effects. The potential allergenicity of the PMI protein has been assessed, and the EFSA GMO Panel considers that the evidence combining *in-vitro* digestibility assays, bioinformatic studies and negative *in-vitro* serum binding tests, that there are no indications that PMI would be an allergen.

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			the possible allergenic potential of this protein a thorough evaluation of its presence in various feed and food fractions is absolutely necessary.	
Germany	Federal Office of Consumer Protection and Food Safety	D, 07.08 Toxicology	Comments by the Federal Agency for Nature Conservation: The study fulfils the requirements of OECD test guideline 408 (90 d rodent study) which is designed to test chemicals. A guideline modified to account for the testing of GMO plant material has not been developed so far. Pusztai (Pusztai et al.; 2003; Genetically Modified Foods: Potential Human Health Effects. Food Safety: Contaminants and Toxins, pp. 347-372) proposed a number of amendments for GMO-feeding studies including comparable start body weight of the animals (varying no more than 5%) and the analysis of organ weight from gut and intestines because those organs come in direct contact with the GMO. For this reason we strongly recommend the development of a GMO specific guideline. The study gives some indications for possible adverse effects of MIR604-maize. Statistical significant changes were observed, which, even though they didn't occur in both genders and both GMO-maize-fed-groups, may show possible adverse effects. Noticeable is the partly significant lower food consumption of the male rats in both GMO-maize-fed-groups during the whole test, which leads to a significant lower increase in body weights in the group of 10% GMO-maize-fed male rats. Together with other results, especially the significant changes in the haemogram of male rats in the group which was fed with 10% GMO-maize thus	The EFSA GMO Panel is of the opinion that OECD test guideline 408 adapted for whole food testing is appropriate to assess safety of GM foods and feeds (as indicated 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 (EFSA, 2006a)). According to this guideline, histological examination of the gastrointestinal tract is part of the toxicological evaluation. In addition, a working group of the EFSA GMO Panel has published a special report on animal testing of GMOs (EFSA, 2008). In summary, the EFSA GMO Panel is satisfied with the way that the subchronic rodent study has been carried out. The other assays performed on the transgenic proteins, such as bioinformatic and *in-vitro* digestibility assays, neither do raise concerns regarding potential toxicity of these proteins. As regards the differences observed in haematological values and organ weights, the EFSA GMO Panel has received additional data on the historic background ranges of these values, based upon which it concluded that most of the individual values of the parameters showing differences fell into the background

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			can give a hint to possible adverse effects of MIR604 maize on the health of the test animals. As a consequence a subsequent feeding study should be requested to address the above uncertainties. The study should cover a longer exposure preferably over two generations to test for chronic effects. In order to conduct this study, the recommended methods given in Pusztai et al. (2003) should be considered.	range. Also no macroscopic and microscopic pathological findings possibly associated to these differences were noted. For body weights, historical background data of rats fed 10% maize diets are provided in annex G of the report on the subchronic rat study. These issues are discussed in more detail in the scientific opinion of the EFSA GMO Panel.
Germany	Federal Office of Consumer Protection and Food Safety	D, 07.08 Toxicology	An acute oral mouse toxicity study was performed for mCry3A protein to confirm the safety of this newly expressed protein. The test was conducted with mCry3A protein produced by micro-organisms. The structural, biochemical and functional equivalence of the microbial substitute to the plant expressed protein should be demonstrated in a study presented in Appendix VII of the application. However Figures 1. and 2. show a slightly higher molecular weight band for the E. coli derived protein compared to the maize derived protein. This observation is not mentioned in the corresponding discussion of the results in App. VII, but is confirmed by results presented in Appendix XVI. The E.coli derived mCry3A substance is a mixture of two proteins of 67 kDa and 69 kDa in a ratio of 2:3. Furthermore the maize derived mCry3A test substance consists to a substantial portion of, according to the applicant, a breakdown product of the 67 kDa mCry3A protein. The German CA does not share the opinion of the applicant that the structural, biochemical and functional equivalence of the two test substances could be clearly demonstrated. The applicant is requested to discuss	The EFSA GMO Panel has received additional information on the identity of the protein bands observed during Western blotting of mCry3A, including data from mass spectrometric analysis of peptides derived from the proteins in the bands. For example, mass spectrometry has been carried out on transgenic mCry3A proteins and the lower MW band has thus been identified as a C-terminal fragment of mCry3A. The microbiologically produced analogue of mCry3A contains two proteins, one of which has an N-terminal extension. These recombinant analogues have retained their functional activities, though, as evidenced by insect bioassays for the two separate forms of mCry3A. In addition, electrophoretic, mass spectrometric, immunoblotting, and glycosylation studies have been carried out with the mCry3A proteins. The EFSA GMO Panel considers that the plant-expressed mCry3A protein and microbiologically produced analogues are similar and that the N-terminal extension is unlikely to have impacted on the functional properties of this protein.

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			the validity of acute oral mouse toxicity study in light of the observed differences of the test substances extracted form E.coli and MIR604 maize. A demonstration of the structural equivalence, by peptide mapping of mCry3A protein and the breakdown product from maize event MIR604 is suggested. In the 90-day rat feeding study MIR604 maize in the diet resulted in consistently lower food consumption in male rats in the 10% and 41,5% GMP-positive Group. The effect of lower food consumption was also observed in female rats but to a lower extent. However the difference between groups in overall consumption of maize, based on grams per day and kg bodyweight, was negligible. It would be desirable to identify the reasons for the observed effect of lower food consumption in the GM-maize fed groups and to have information on the food consumption of the test groups in the pre-test phase. Furthermore several parameters of haematology, blood clinical chemistry and organ weights show statistically significant differences compared with the control groups. However no one of the observed effects occurred in both sexes or was dose dependent. Histopathological examinations of organs and tissue revealed no relevant effects. Most effects occurred in the lower dosed groups. The author of the 90-days rat feeding study concludes that the observed effects are not related to the administration of MIR604 maize in the diet. After careful consideration of the supplied data, the national competent authority of Germany sees no sound evidence to reject the conclusion of the author.	For body weights, historical background data of rats fed 10% maize diets are provided in annex G of the report on the subchronic rat study, pages 105-112 of appendix IX. As regards the differences observed in haematological values and organ weights, the EFSA GMO Panel has received additional data on the historic background ranges of these values, based upon which it concluded that most of the individual values of the parameters showing differences fell into the background range. No macroscopic and microscopic pathological findings were noted that these differences could be associated with either. These issues are discussed in more detail in the scientific opinion of the EFSA GMO Panel.

Country	Organisation	Reference	Comments	EFSA GMO Panel responses
			Nevertheless it would be desirable to get information if the observed results are within the range of historical control data.	
Austria	Ministry of Health and Women	D, 07.09 Allergenicity	The evaluation of the substantial equivalence of the microbial substitute for the PMI protein and the plant-derived protein shows that the plant derived protein has a slightly lower molecular weight than the PMI protein produced by E. coli (Appendix II). Additionally, the enzymatic activities of the leaf extracts show a large variation in the specific activity of the enzyme although only three leaves were tested while the activity of the microbially produced enzyme was much lower. For a thorough protein characterisation and comparative analysis a fingerprinting method based on mass spectrometry or similar methods are recommended. Additionally, the evaluation of the amino acid homology of the PMI marker with known allergens revealed a match of 8 identical amino acids between the plant PMI protein and an allergen from Rana sp. Evaluation of the protein for reactivity with serum IgE from allergic patients revealed that one of the PMI proteins tested was recognized. Therefore further studies should evaluate the potential for allergenicity of the PMI marker protein. In the dossier the notifier writes:"The results of these analyses revealed that there was no significant similarity between any of the sequential MIR604 PMI 80-amino acid peptides and any entries in the SBI Allergen Database. A separate search for sequence homology at the level of eight or more consecutive amino acids revealed a	Based on the data provided by the applicant, it was noted that the microbiologically produced analogue of PMI contains an N-terminal extension. This microbiologically produced analogue has retained its functional activity as evidenced by enzymatic assays for PMI. The EFSA GMO Panel recognizes that enzymes and insecticidal proteins expressed in plant materials like leaves are naturally characterized by variation of their biological activity, (e.g. influences of matrix components). Taking this variation into account and the fact that the values measured by the applicant are well within the same order of magnitude, plus the other data including immunoblotting, and glycosylation assays, the EFSA GMO Panel therefore considers that the plant-expressed transgenic protein and microbiologically produced analogue are similar. As regards the 8-amino-acid stretch shared with a frog leg allergen, the report describing this allergen in scientific literature was based on the immune reaction of a single patient. The applicant has thus contacted the authors of the article, which have tested the binding of the isolated IgE-serum to the PMI protein and to the frog allergen as positive control. No reaction with PMI has thus been observed. Whereas it is

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			single match of eight identical amino acids between MIR604 PMI and a recently identified allergen, a-parvalbumin". In this context it has to be stated that the WHO/FAO [Evaluation of Allergenicity of Genetically Modified Foods, Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology,22 – 25 January 2001] recommend an allergological assessment already for six identical amino acids.	realized that other authors have made recommendations regarding the number of sera to be used for obtaining reliable data regarding IgE-binding, the EFSA GMO Panel considers that the applicant has done as much as it was possible within its capacity. As regards the minimum size of the identical stretch that should be considered in the bioinformatic studies comparing the transgenic protein with allergens, the FAO/WHO 2001 consultation cited by the member state has served as input for the Codex alimentarius guideline for safety assessment of foods derived from GM plants. These guidelines do not specifically recommend a minimum threshold, whereas it was realized that previous publications have recommended thresholds of 6 or 8 contiguous amino acids. Similar to Codex, it is noted that choosing 6 amino acids is likely to yield higher numbers of false positive outcomes, as has also been confirmed in literature (e.g., Fiers et al., 2004).
Spain	Ministry of the Environment	D, 07.09 Allergenicity | D, 12.03 General Surveillance of the impact of the GM plant	EFSA/GMO/UK/2005/11 1) Allergenicity The document is mentioning since both proteins are from a bacterial source no allergenic effects should be expected. This is not totally accurate since the proteins used have been modified (mCry3A instead of Cry3A and modified PMI protein for its expression in plants) Sequences of comparison of 8 amino acids are used, and this facilitates the existence of false negatives. It does not apply the criterion FAO-OMS of 2001 (comparison of 6 amino	1) The sequences stored by Syngenta in its database have been obtained from publicly available protein sequence databases like SwissProt. Selection of these sequences has been done based on their identification as allergens by the sequence accession records or by listings of allergens from well-known sources (WHO-IUIS, SwissProt allergen list, and Farrp database). Various tests have been performed comparing the plant-expressed and microbiologically produced

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			acids), which offers major sanitary safety. It has been used an internal data base of information of Syngenta to compare sequence homologies between the expressed proteins and known allergens and a computer program performed by the company, of which one does not give any more information. There are public data bases of information and programs of comparison of accessible sequences, so it is unclear why they have chosen this procedure. Anyway, we consider that a comparison also with the public data bases is also needed to contribute to a further transparency. It seems that the protein PMI has sequences similar to an allergenic parvoalbumine, and it has been done a serologic assay. But they have not used really the PMI protein expressed in the plant but just the obtained by E. coli, with 2 different amino acids and an extra of 16 amino acids in the N border, which questions the validity of the result. For this specific serologic test just one serum has been used for the serum screening analysis. We consider that this is not statistically significant. Even for high quality serums (with Ig E's high levels) from patients with documented record of allergy to this protein, and with a confidence level of only 95 %, at least 6 different serums would be needed. Regarding in vitro digestion,it is said that after 2 minutes in gastrointestinal fluid nor intact PMI and mCry3A neither "immunoreactive" fragments proteins were detected. Since it has not been verified if the fragments are immunoreactive or not), it does not seem appropriate to make such an affirmation. 2) Monitoring plan This application is	forms of PMI. Despite the two amino acids difference and the additional N-terminal extension of the microbiologically produced PMI analogue, the latter was still functional and was similar to its plant-expressed counterpart in enzyme activity and lack of glycosylation. 3D-Modelling of the structure of PMI performed by the EFSA GMO Panel did not indicate any major changes that the two different amino acids may cause. The 8-amino-acid stretch that was identical between PMI and the frog leg allergen does not contain any of the mutated amino acids. It is therefore not suspected to impact on the binding assay with IgE from the frog allergen patient As regards the number of sera that were reactive against the frog leg allergen, the report that had described this allergen in scientific literature was based on the immune reaction of a single patient. The applicant has thus contacted the authors of the article, which have tested the binding of the isolated IgE-serum to the PMI protein and to the frog allergen as positive control. No reaction with PMI has thus been observed. Whereas it is realized that other authors have made recommendations regarding the number of sera to be used for obtaining reliable data regarding IgE-binding, the EFSA GMO Panel considers that the applicant has done as much as has been possible within its capacity. As regards the minimum size of the identical stretch that should be considered in the

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			only for authorisation for food and feed uses, and not for cultivation. In any case, measures to avoid accidental spillage should be strengthened. The general surveillance plan under the monitoring plan must assure these aspects.	bioinformatic studies comparing the transgenic protein with allergens, the FAO/WHO 2001 consultation cited by the member state has served as input for the Codex alimentarius guideline for safety assessment of foods derived from GM plants. These guidelines do not specifically recommend a minimum threshold, whereas it was realized that previous publications have recommended thresholds of 6 or 8 contiguous amino acids. Similar to Codex, it is noted that choosing 6 amino acids is likely to yield higher numbers of false positive outcomes, as has also been confirmed in literature (e.g., Fiers et al., 2004). 2) The EFSA GMO 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. The applicant provided a '*Monitoring plan for the import and use of GM maize event MIR604 in the EU*' according to Annex VII of Directive 2001/18/EC, including a methodology for the general surveillance of viable maize MIR604. See section 6.1.2. of the scientific opinion In addition, see section 5.2 of the PMEM opinion (EFSA, 2006b):

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				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 (…).
Austria	Ministry of Health and Women	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 regarding the use for human consumption should be added to the dossier.	See section 5.1.6 of the scientific opinion: *The risk assessment concluded that no data have emerged to indicate that maize MIR604 is any less safe than its non-GM comparator. In addition, no biologically relevant agronomic and compositional changes were identified in maize MIR604. Therefore, in line with the guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.*
Germany	Federal Office of Consumer Protection and Food Safety	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Comments by the Federal Agency for Nature Conservation: An important part of the environmental risk assessment is to assess the possible exposition of the environment with the GMP. For this application the main exposition will	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the

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			be through loss and spillage. Therefore information about the way of introduction of the genetically modified maize MIR604 to the EU market, including the expected amount of import, should be provided. Furthermore the possibility of unintended release via spillage during transportation and processing, demands data about the kind of transportation and the way of reloading. In addition pathways of environmental exposure during the production process need to be mentioned.	cultivation of maize MIR604. See section 6.1.2 of the scientific opinion
Sweden	Swedish Board of Agriculture	D, 10 Potential changes in the interactions of the GM plant with the biotic…	We have identified a risk that has not been addressed in this notification. If some of the Bt-toxin is not degraded during the passage through the gastrointestinal tract of animals that are fed this maize, Bt-toxin may appear in the manure. The Bt-toxin is targeted against insects in the order coleoptera. There are species in the order coleoptera that feed on manure. If such species are sensitive to this particular Bt-toxin, they may be negatively affected by the release of maize MIR 604. The applicant has shown that the Bt-toxin is degraded in simulated gastric fluid, and that it is inactivated by heat treatment. However, it is not shown that the proteins are degraded in vivo. Moreover, it is not shown that coprophagous coleopteran species are not negatively affected by the Bt-toxin. This is not known, especially in the light of the altered specificity of the toxin compared to the native Cry3A and the absence of test for the mCry3A´s specificity towards different insect species. We propose that this risk should be assessed. If necessary, there should be case specific monitoring	See section 6.1.1.4 of the scientific opinion: *The EFSA GMO Panel assessed whether the Cry3A protein might potentially affect non-target organisms by entering the environment in manure and faeces from the gastrointestinal tracts of animals fed maize MIR604.* *Data supplied by the applicant suggest that only small amounts of the modified Cry3A protein enter the environment due to low expression in kernels. Moreover, most Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only low amounts of Cry proteins would remain intact to pass out in faeces (e.g., Einspanier et al., 2004; Lutz et al., 2005, 2006; Wiedemann et al., 2006; Guertler et al., 2008). There would subsequently be further degradation of the Cry proteins in the manure and faeces due to microbial processes.* *Exposure of soil and water environments to this Cry protein from disposal of animal wastes or*

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			for this risk.	*accidental spillage of maize kernels is likely to be very low and localized. While Cry proteins can bind to clay minerals and humic substances in soil, thereby reducing their availability to microorganisms for degradation, a number of studies revealed that there is no persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008).* *Considering the scope of the application that excludes cultivation and the intended uses of maize MIR604, it can be concluded that the exposure of potentially sensitive non-target organisms (e.g., coprophagous Coleoptera species) to the Cry3A protein is likely to be very low and of no biological relevance.*
Austria	Ministry of Health and Women	D, 10.04 Interactions between the GM plant and target organisms	Mechanism of interaction between the GM plant and target organisms The applicant of the transgenic maize event MIR604 has made changes of the sequence of the native cry3A gene derived from Bacillus thuringiensis subsp. tenebrionis in order to enhance the activity of this toxin against the western corn rootworm Diabrotica v. virgifera and other related pests. Although a description of the native Cry3A toxin is given (Sekar et al. 1987), the study describing the mode of action of the modified Cry3A toxin is not added to the application [Chen, E. & Stacy, C. (2003). Modified Cry3A toxins and nucleic acid sequences coding therefore. WO Patent No. 03/018810.] and therefore the exact mode of action as well as the changed specificity spectrum of this	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604.

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			toxin is unclear. Since the native toxin is specific to chrysomelid pests, at least the effects on other Chrysomelidae should have been evaluated.	
Norway	Directorate for Nature Management	D, 10.04 Interactions between the GM plant and target organisms	Use of the cry3A gene in MIR604 leads to the target organisms being continuously exposed to the Bt-toxin, as opposed to spraying where the target insects are only exposed at the time of spraying (and a limited time thereafter). Information regarding development of resistance in the target pests to the CRY3A protein, followed by a change to less environmentally safe pesticides or increased doses & number of applications, is necessary to assess the impact of MIR604 on the environment, its contribution to a sustainable development plus the benefit to society of the maize line.	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604. Sustainability-related issues fall outside the remit of the EFSA GMO Panel. Furthermore human and animal health issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the EFSA GMO Panel.
Austria	Ministry of Health and Women	D, 10.05 Interactions of the GM plant with non-target organisms	1) Since import, transport and processing of MIR604 maize may result in the presence of accidental occurrence or release of MIR604 maize in the environment, potential adverse effects of the GM maize on non-target organisms must be considered. Since MIR604 maize disposes of two new proteins the potential environmental effects of both newly expressed proteins must be examined. 2) As expected with a root specific promoter expression of mCry3A is reported to be high in roots of MIR604. Even higher expression levels of mCry3A were recorded in leaf	1) The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604. The Environmental Risk Assessment (ERA) does not indicate any environmental negative effects due to accidental spillage of imported seeds. 2) Variations in the levels of gene expression (such as those observed here in the

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			tissues of MIR604 hybrids. For example, at seed maturity the hybrid MIR604B had 3 times higher protein levels in leaves compared to the roots (on a fresh weight basis, Appendix III). Although expression levels of the PMI protein were relatively low compared to mCry3A protein levels in several tissues, highest expression levels for the PMI protein were recorded in pollen (1,9-2,6 ìg/g fresh weight, Appendix III). These high protein levels might have consequences for leaf-feeding or pollen feeding non-target organisms and should be subject to further evaluation. However, the agronomic performance analysis of MIR604 provided by the applicant only considered pest species such as Western and Northern Corn Rootworm as well as yield evaluation and susceptibility to other maize pathogens such as northern corn leaf blight etc. (see Appendix CBI.4). No effects on non-target organisms have been evaluated so far. As the native Cry3A toxin is active against Chrysomelidae, as a minimum requirement adverse effects on non-target Chrysomelidae should be included in the risk assessment.	kernels) are not uncommon in plants.
Denmark	Danish Forest and Nature Agency	D, 10.05 Interactions of the GM plant with non-target organisms	Data and information on consequences to non-target organisms such as herbivores and predators should be present.	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604. The ERA does not

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				indicate any environmental negative effects due to accidental spillage of imported seeds.
Norway	Directorate for Nature Management	D, 10.05 Interactions of the GM plant with non-target organisms	Although a root specific promoter has been used in the cry-gene construct (possibly to limit the exposure of the CRY3A protein to the environment and target the root-damaging pest species) the Notifier states in 3a of Part I (Technical details) that quantifiable levels of the CRY3A protein was found in all parts of the MIR604 plants, except in the pollen. The highest levels of CRY3A was found in the leaves of MIR604 (both fresh and dry weight) and thereafter comes roots and whole plants. Since the whole plant expresses the protein we need to know what research has been done on the impact of this specific Bt-toxin on non-target species (both soil-living and otherwise). Although the notification does not cover cultivation in the EU and EFTA countries, there is a risk that non-target organisms will consume MIR604 in areas of cultivation. The information is also relevant in case of accidental spillage of MIR604in the EU/EFTA where seed, or volunteer plants, are consumed directly by non-target organisms.	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation. The ERA does not indicate any environmental negative effects due to accidental spillage of imported seeds.
Norway	Directorate for Nature Management	D, 10.09 Impacts of the specific cultivation, management and harvesting…	The genetically modified MIR604 is resistant to certain insect pests in the order Coleoptera. Use of the cry3A gene in the maize line will expectedly lead to changes in cultivation practices (e.g. use of pesticides) compared to conventional maize. Information regarding use of insecticides (changes in volumes and types of chemicals) is necessary to	The scope of the application is for food and feed uses, import and processing of maize MIR604 and excludes cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation of maize MIR604.

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			assess the impact of the genetic modification on the environment, as well as socioeconomic effects of the changes, and this should be further discussed by the Notifier.	Furthermore human and animal health issues related to plant-protection products as well as potential socio-economic effects are regulated by Directive 91/414/EEC and fall outside the remit of the EFSA GMO Panel.
Germany	Federal Office of Consumer Protection and Food Safety	D, 11 Potential interactions with the abiotic environment	Comments by the Federal Agency for Nature Conservation: A general surveillance plan that meets the scope of the application (food, feed, import and processing) is required. Amongst others, the general surveillance plan has to focus on possible pathways how Event MIR604 maize can enter the environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal of the GMO. Therefore, the applicant is requested to provide an appropriate monitoring plan to observe the spread, persistence and accumulation of the inserted genetic sequences and the corresponding proteins in organisms and environmental media (soil, air, water). The monitoring plan has to meet the following requirements: – A fully specified list of monitoring parameters has to be defined. For each parameter a detailed statement of the parameter definition, of the observation methods (collection and analysis of samples with references), of the frequencies of observations (time and number of visits to collect data) and of the locations (habitat type, number, size and spatial arrangement of plots) has to be presented. – An operating schedule giving full details of points in time, places and monitoring parameters to be observed should be requested from the applicant. – Representativeness and local	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 supplied by the applicant is in line with this guidance. The applicant provided a '*Monitoring plan for the import and use of GM maize event MIR604 in the EU*' according to Annex VII of Directive 2001/18/EC, including a methodology for the general surveillance of viable maize MIR604. See section 6.1.2. of the scientific opinion In addition, 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*

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			adaptation of the monitoring is required. The concept of sampling needs to be elaborated. Particularly, it must be explained how the necessary representativeness of the collected data in space and time is ascertained. – It must be stated, how the condition of the environment before the placing on the market is described (determination of the baseline status of the receiving environment as a point of reference). – The methods of data analysis including the statistical methods have to be elaborated in full detail. – Use of external people and existing networks: The monitoring expertise of external people involved in the monitoring activities and detailed information about participating networks (e.g. name, EU country, responsible authority, availability, scope and composition) have to be specified. In case that monitoring data are 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 submission of complete reports on the fully detailed monitoring results on an annual basis. All raw data have to be appended to the reports. – It has to be stated how the results of the monitoring will be published.	*Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).*
Austria	Ministry of Health and Women	D, 12 Environmental Monitoring Plan	The monitoring plan for MIR604 maize provided by the applicant can be considered as rather superficial. It lacks details of the arrangement for carrying out the general surveillance and lacks the description of specific criteria, routes of observance, procedures etc. related with the notified product and its intended	See response to the previous German comment on D,11

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			uses. Among others, the following questions remain unanswered: „X It is unclear what data will be collected. „X How will the applicant collect and scientifically evaluate and report ¡§reliable scientific evidence¡¨. „X Which ¡§networks¡¨ will be involved and what is the ¡§common industry approach¡¨ (technical dossier, Appendix XXII, p 4)? „X Who is responsible for choosing relevant persons? „X Which criteria do these people have to fulfil? What kind of knowledge or expertise is required? „X What are the criteria for different intensities of surveillance activities in different EU countries and for the choice of ¡§representative areas¡¨ for general observation (Appendix XXII, p 5)? „X How are traders or processors of maize grains supposed to assess any potential adverse effects on the environment or human health? „X Who is responsible of providing traders and processors with the relevant information? „X Who is responsible of informing the European feed industry? According to Annex VII, C.5 of directive 2001/18/EG it is necessary that the design of the monitoring plan ¡§[identifies] who (notifier, users) will carry out the various tasks [¡K] and who is responsible for ensuring that the monitoring plan is set into place and carried out appropriate [¡K]¡¨ „X How are cooperation and information flow as well as communication between involved people/institutions organized? „X Which entities/consumer groups should be monitored particularly? To ensure comparability of any data, observations or any kind of information processes as well as reporting tools etc. should be standardized for the general	

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			surveillance. As the responsibility remains with the notifier appropriate suggestions should be submitted. Post-market surveillance and monitoring of MIR604 should be carried out not only passively, as suggested by the applicant, but rather actively, with particular population segments being identified and followed actively over a period and specific health endpoints as well as consumption profiles being measured in order to be able to detect any unintended effects. The applicant intends further evaluation of an adverse effects only ¡§¡Kwhere there is scientifically valid evidence of a potential adverse effect linked to the genetic modification¡¨ (Appendix XXII, p 5). On the other hand, the applicant admits that ¡§¡K. it will be difficult to apply classical scientific methods to general surveillance¡¨ (p 4). These statements contradict each other. General surveillance should on first hand provide and organize data with the aim of establishing a correlation rather than causality between the observations and the data associated which will give indications of potential adverse effects and will trigger further studies to evaluate a possible causal link to the GMO. Additionally, the monitoring plan for the MIR604 maize does not recognize accidental spillage and release of MIR604 maize. It should incorporate specific risk management strategies in case an unintentional release occurs as well as foresee measures for monitoring and management of accidental spilling or release. Experience as well as results of surveys and inspections in Austria have shown unintended or	

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			technically unavoidable contamination (e.g. through transport and processing) leading to the unintended release of seed or grains. Although the risk from unintentional release of the maize e.g. via spilling is recognized by the applicant no specific procedures for detection and measures for removal are proposed.	
Sweden	Swedish Board of Agriculture	D, 12 Environmental Monitoring Plan	The plan for general surveillance needs to be supplemented with more details. It is not clear who will be involved, what information will be collected and how participation will be ensured. The plan for general surveillance should be comparable with the requirements on the monitoring plans in already approved notifications concerning import of genetically modified maize under directive 2001/18/EC. Relevant decisions are 2004/643/EC (NK 603), 2005/608/EC (MON 863) and 2005/772/EG (1507). It may be considered to include case specific monitoring of the risk for negative effects on certain species of coprophagous coleoptera (see comment on the environmental risk assessment).	See response to the previous German comment on D,11
Norway	Directorate for Nature Management	D, 12.03 General Surveillance of the impact of the GM plant	In appendix XXII of part I, a monitoring plan for the import and use of genetically modified maize event MIR604 in the EU is proposed. The outline of the plan is good, but at the same time very broad and we would request that the Notifier: a) Provide a list of persons/organisations that will be responsible for the general surveillance. Consent for marketing of MIR604 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	See response to the previous German comment on D,11

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			participants. The Notifier has a good point that all areas of the EU/EFTA probably do not need the same level of surveillance and the list over chosen participants should reflect this. Furthermore, the Notifier should choose some of the participants to perform more "active monitoring" and these should be chosen in areas of greatest activity (largest import and transport from entry points) and from areas that are more environmentally vulnerable (with maize cultivation, endangered non-target species vulnerable to the CRY3A protein etc) b) Provide a more detailed outline of key points that the general surveillance will address and that will be reported to the Notifier. Examples of points that should be reported are: · An overview of the amount of the genetically modified maize line that was imported to each of the receiving countries during the previous year and the main uses of the imported GM maize. · A summary of the active monitoring that was carried out by the Notifier during the previous year, and the results of this monitoring activity (no findings should also be reported as it shows that an active monitoring has been performed) · An overview of the activities carried out by the Notifier to supply technical information, recommendations etc to operators, end-users etc · The actions taken to get feed-back from key stakeholders (those that do not perform the "active monitoring"), and a summary of the information that was gathered.	

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Norway	Directorate for Nature Management	D, 12.05 Implementing General Surveillance	We agree with the Notifier that persons/organisations normally involved in the import and use of the maize will be in the best position to participate in a general surveillance plan (appendix XXII section 1.1.2). However, a plan of such relevant participants should be in place before consent to market MIR604 is given, to ensure that they are willing to undertake the job in question. We support the Notifier with regards to the comment that the same level of participation is probably not needed in all EU/EFTA countries, but should vary with the extent of import, processing, transport etc. In addition we would say that the intensity should also vary according to the "environmentally vulnerability" of the region. Does the region cultivate maize? What is the status of non-target species (endangered species that are receptive to the CRY3A protein)? These are important aspects that should be taken into consideration and be reflected in the surveillance plan, and with regards to the choice of and number of participants.	See response to the previous German comment on D,11
Norway	Directorate for Nature Management	D, 12.06 Reporting the results of monitoring	As requested in D, 12.03 the Notifier should provide a more detailed outline of key points to be addressed by the general surveillance and included in the monitoring reports from the Notifier. Examples of points that should be reported are: · An overview of the amount of the genetically modified maize line that was imported to each of the receiving countries during the previous year and the main uses of the imported GM maize. · A summary of the active monitoring that was carried out by the Notifier during the previous year, and the results of this	See response to the previous German comment on D,11

Application EFSA-GMO-UK-2005-11 (Maize MIR604) **ANNEX G** **Comments and opinions submitted by Member States during the three-month consultation period**				
Country	**Organisation**	**Reference**	**Comments**	**EFSA GMO Panel responses**
			monitoring activity (no findings should also be reported as it shows that an active monitoring has been performed) · An overview of the activities carried out by the Notifier to supply technical information, recommendations etc to operators, end-users etc · The actions taken to get feed-back from key stakeholders (those that do not perform the "active monitoring"), and a summary of the information that was gathered.	
				Comments from other EFSAnet users
Greece	Hellenic Food Authority	General comments	It is remarkable that the Technical Dossier does not contain a review of the experiments performed in order to support the different key component parts of the application. This does not enable the competent bodies to understand the extent of substantiation of the application.	The risk assessment carried out by the EFSA GMO Panel and detailed in the scientific opinion is based on the information provided in the application EFSA-GMO-UK-2005-11 relating to maize MIR604 submitted in the EU, including additional information from the applicant, and scientific comments that were raised by Member States. See the conclusions of the EFSA GMO Panel in the section '*Overall Conclusions and Recommendations*'
The Nether-lands	Ministry of agriculture	C, 03 Size, source (name) of donor organism(s) and intended function of each…	General question (also pertaining to molecular characterization and potential environmental effects) Code: C.3, Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion. It is generally considered that the micro-organism Bacillus thuringiensis are ubiquitously present in the environment and that	The mutations that have occurred in the mCry3A protein are described in the section on molecular characterization of the scientific opinion of the EFSA GMO Panel (see section 3.1.2). In order to create a cleavage site for cathepsin, amino acid residues number 48 (N-terminal methionine) and 155-157 have been substituted. The outcome of

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			derived pesticide preparations are considered safe for humans. In addition, it is known that the insectidical crystal (Cry) proteins derived from parasporal inclusions of B. thuringiensis act specifically on particular insect species, and not on humans or domestic animals. If changes are made to the Cry proteins inserted in transgenic plants, it is important to have insight into the nature of these changes and whether they may affect the potential toxicity and allergenicity for human and animal consumers. The amino acid sequence of the Cry3A protein has been modified such that the protein became cleavable by cathepsin G. No data are apparently provided on the details of this modification, such as the amino acids that have been mutated. The applicant should be requested to provide these details and whether this change may also have its implications for human and animal consumers.	the various stability-, toxicity-, and allergenicity-assays, including bioinformatic, *in-vitro* digestibility, thermal stability, presence in processing fractions, acute toxicity, and whole-product subchronic toxicity do not indicate any adverse effects that might emanate from the expression of the *mCry3A* gene in maize MIR604.
Greece	Hellenic Food Authority	D, 02 Information on the sequences actually inserted or deleted	According to BLASTN analysis of the flanking sequence from both 5' and 3' regions of the MIR604 shows no significant homology with any known Zea mays sequences. For this reason it is concluded that the insertion of the expression cassette in MIR604 has occurred in a region of the Zea mays genome that is not well annotated and that the insert does not disrupt any known endogenous Zea mays gene. Therefore, the sequence analysis of the 5' and 3' flanking regions of the insert should be continued until a known genomic region of the host plant is found.	See section 3.1.2 of the scientific opinion: *Sequences flanking the 5' and 3' regions of the MIR604 event have been determined, extending at least 1 Kb into the host genome. A recent (2008) BLASTN analysis of the 5' and 3' flanking sequences show no significant homology with any known Zea mays sequences. ORF analysis of all six potential reading frames at both the 5' and 3' flanking regions revealed the presence of one putative novel ORF. This is 258 bp in length, begins in the NOS terminator and extends through the T-DNA into the 3' flanking sequence. The ~240 bp upstream of this putative ORF is terminator sequence, and no promoter elements*

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				have been found. Therefore, transcription of this putative ORF is unlikely. In the unlikely event that the ORF were to be transcribed, bioinformatic analysis indicates no sequence homologies to known toxins or allergens.
Greece	Hellenic Food Authority	D, 03 Information on the expression of the insert	According to the dossier, bioinformatics analysis did not give evidence that a novel transcript might arise at either junction of the insert. However, an experimental confirmation through transcriptional analysis should be carried out to confirm the theoretical bioinformatics analysis.	ORF analysis of all six potential reading frames at both the 5' and 3' flanking regions revealed the presence of one putative novel ORF. This is 258 bp in length, begins in the NOS terminator and extends through the T-DNA into the 3' flanking sequence. The ~240 bp upstream of this putative ORF is terminator sequence, and no promoter elements have been found. Therefore, transcription of this putative ORF is unlikely. In the unlikely event that the ORF were to be transcribed, bioinformatic analysis indicate no sequence homologies to known toxins or allergens.
The Nether-lands	Ministry of agriculture	D, 07.03 Selection of compounds for analysis	Question on the comparative assessment (safety issue) Code: D.7.3, Selection of material and compounds for analysis According to the guidance of the EFSA GMO Panel, compositional analysis should include samples from multiple seasons and locations, taking these factors into account in the statistical analysis. Please note that the establishment of equivalence is in fact the starting point of the comparative safety assessment. Therefore the conclusions based upon the compositional data, including key antinutrients, should be based on sufficient data. The antinutrient phytic acid and a number of other analytes have been measured in only one year. The applicant should be requested to	No differences in phytic acid have been observed between GM and non GM maize. Therefore, there are no indications that would necessitate further exploration of differences in phytic acid content. The EFSA GMO Panel considers that the overall data set that has been provided for compositional analysis is sufficient.

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			provide compositional data from an additional year for those key components for which it has only provided data from one year, such as phytic acid. It is therefore not possible to verify whether the occurrence or not of particular differences is consistent between seasons, which would increase the validness of conclusions regarding the compositional equivalence of maize 59122 to its conventional counterpart.	
Norway	Norwegian Scientific Committee for Food safety	D, 07.08 Toxicology	7.8.4 Testing of the whole GM food/feed The bodyweights in the male group fed diet containing 10 % MIR604mCry3A were lower than the control group fed maize without mCry3A. Also, food consumption in males fed diet containing 10 % MIR604mCry3A and 41.5 % MIR604mCry3A were lower throughout the study compared to respective controls. The GMO Panel of the Norwegian Scientific Committee for Food Safety is of the opinion that the applicant must make a clearer (or elucidate) explanation why rats consume less mCry3A maize than rats eating maize without mCry3A.	The EFSA GMO Panel has taken notice of the decreased consumption in males on 41.5% GM diet lower in weeks 1, 2, 3, 5, and 6, and in females on both GM diets in week 2. The EFSA GMO Panel also mentions the observed decrease in bodyweights in males fed diets containing 10% MIR604 maize in its scientific opinion. Differences in bodyweights were only transiently observed in other groups fed diets containing 10% (female) and 41.5% (male, female) GM maize. There is therefore neither an apparent consistency nor a dose-response relationship in these effects. For body weights, historical background data of rats fed 10% maize diets are provided in annex G.
The Nether-lands	Ministry of agriculture	D, 07.08 Toxicology | D, 07.09 Allergenicity	Question pertaining both to safety testing and to molecular characterization Codes: D.7.8.1, 1) Safety assessment of newly expressed proteins; and D.7.9.1, Assessment of allergenicity of the newly expressed protein For the interpretation of the results of safety experiments with purified transgenic proteins and their extrapolation to the safety of consumption of MIR604, it is important that the purified transgenic	1-3) The EFSA GMO Panel has received additional information on the identity of the protein bands observed during Western blotting of mCry3A. For example, mass spectrometry has been carried out on transgenic mCry3A proteins and the lower MW band has thus been identified as a C-terminal fragment of mCry3A. The microbiologically produced analogue of mCry3A

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			proteins are representative for the proteins present in consumed products derived from genetically modified maize MIR604. This requirement is also put forward in the EFSA GMO Panel's guidance document. 2) The recombinant proteins of Cry3A and PMI produced in bacteria and used for safety testing contained additional N-terminal extensions that were not present in the plant-expressed counterparts. In addition, apparently intact (67.7 kDa) and degraded (55 kDa) forms of the Cry3A protein occur in MIR604 maize. Plant-expressed PMI also contains two amino acid substitutions that do not occur in the bacterially expressed PMI. The applicant should indicate more in detail what the identity of the 55-kDa degradation product of Cry3A is. 3) The applicant should also indicate how the differences in sequences between the plant- and bacterially expressed proteins might affect the toxicity and allergenicity potential of the Cry3A and PMI proteins to humans and animals.	contains two proteins, one of which has an N-terminal extension. The microbiologically produced PMI protein also contains an N-terminal extension. These microbiologically produced analogues have retained their functional activities, though, as evidenced by insect bioassays for the two separate forms of mCry3A and enzymatic assays of PMI. In addition, electrophoretic, immunoblotting, and glycosylation studies have been carried out with the mCry3A and PMI proteins, as well as mass spectrometric studies with mCry3A. The EFSA GMO Panel considers that the plant-expressed mCry3A and PMI proteins and their microbiologically produced analogues are similar and that the N-terminal extensions are unlikely to have impacted on the functional properties of these proteins.
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 MIR604 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	The issue of potential adjuventicity and immunogenicity of Cry proteins, including Cry3A, is addressed in the scientific opinion of the EFSA GMO Panel. In summary, the absence of IgE responses in the cited articles indicates low or nul allergenic potential. Bioinformatic studies have indicated that allergenicity of Cry proteins is unlikely. In addition, maize is not a common allergenic food, and therefore any adjuvanticity observed only in animal experiments after high doses of Cry proteins does not give rise to concerns regarding potentially

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			likelihood of an increase in allergenic activity due to mCry3A protein in food and feed from maize MIR604 cannot be excluded. Thus, the Panel's view is that as the adjuvant effect of mCry3A with reasonable certainty cannot be excluded, the applicant in relation to a possible adjuvant effect of mCry3A must comment upon the mouse studies showing humoral antibody response of Cry1A proteins. Further, although the mCry3A 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 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	enhanced allergenicity of maize expressing low levels of the Cry proteins. More specifically, after intraperitoneal (i.p.), intranasal (i.n.) or intragastric administration of Cry1Ac and i.p. and i.n. administration of Cry3A to mice at relatively high dosage, IgG, IgM and mucosal IgA response were induced, but no IgE response was observed (Guerrero et al., 2004; Vazquez-Padron et al., 1999; 2000). This demonstrates that Cry1Ac and Cry3A have no allergenic potential under the conditions used. Furthermore, Cry1Ac has been shown to act as an adjuvant e.g. it enhances the mucosal and/or the systemic antibody response to an antigen, i.e. hepatitis B surface antigen or the capsular polysaccharide of *Streptococcus pneumoniae*, which is co-administered with the Cry protein through the i.g., i.p., and i.n. routes (Vazquez et. al., 1999; Moreno-Fierros et al., 2003). The EFSA GMO Panel is of the opinion that as maize is not a common allergenic food, the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration, is unlikely to raise any concerns regarding allergenicity.

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			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.	

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