Application EFSA-GMO-CZ-2005-27 (Maize MON88017) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Austria	Ministry of Health, Family and Youth, Dep. IV/9	General comments	Detection Method The detection method described in the dossier can be regarded as appropriate, nevertheless it should be noted that at the moment the validation process is on step 3 (http://gmo-crl.jrc.it/statusofdoss.htm). Latest before the intended placing on the market the validation of the detection method should be completed and published.	Outside the remit of the GMO Panel
Austria	Ministry of Health, Family and Youth, Dep. IV/9	General comments	Post-market monitoring of GM-food According to Art. 5 (3) k) of EU-Regulation 1829/2003 a post-market monitoring-plan should be added to the dossier.	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 01 Description of the trait(s) and characteristics which have been introduced…	The maize lines used as controls in the molecular characterisation of MON 88017 are insufficiently specified. It is indicated that conventional maize was used for comparisons, however, without giving further details (page 31, technical dossier). The referenced Technical Report by Monsanto concerning molecular characterisation of the inserted traits (Beazley et al. 2002b) indicates that a non-transgenic corn representing the genetic background was used and makes reference to another Technical Report (McCracken et al. 2002). This report however is not annexed to the dossier, therefore it cannot be assessed whether and which details on the nature and origin of control lines are supplied in this report. For an adequate assessment of results sufficient information on the used control substances is required and the necessary information should be specified within the dossier. Likewise the results of the comparison of the flanking sequences bordering the insert in MON 88017 and the respective 260 bp sequence in conventional maize lack sufficient detail. The dossier only states that the respective sequences “share strong similarities” (page 56, technical dossier), without supplying convincing details to clearly demonstrate that these sequences are native to the maize genome. Given the vast amount of sequencing data for maize available, reference should be made whether information is available on the location of the identified flanking sequences in the maize genome and their functional relevance. Furthermore through sequencing border elements unintended modifications were detected (deletions of 25-27 bp compared to PCR clones from conventional maize, as well as an insertion of 20 bp). No information is included in the dossier on the significance	1) The GMO Panel asked for more details on the flanking sequences and the pre-insertion locus. Further analysis performed by the applicant demonstrate that the insertion locus is a single copy sequence located in the nuclear genome of maize, in chromosome 4. 2) The Panel asked for more information on the preinsertion locus. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical, and compositional analyses showed that MON 88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and that the insertion of the transgene *per se* does not pose a safety hazard. 3) The data provided by the applicant is considered sufficient to prove genetic and phenotypic stability over generations.

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			of these modifications and it is not elaborated how theses differences were taken into account for an assessment of unintended effects of the genetic modification. The demonstration of genetic stability by southern blots assessing the gross molecular structure of the inserts in transgenic maize plants of several generations demonstrates that the overall genetic makeup is retained in all examined generations. These results however are not in unambiguous agreement with the data of the assessment of phenotypic stability as described below. For the demonstration of genetic and phenotypic stability these differences have to be sufficiently explained.	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 03 Information on the expression of the insert	The notifier states that the range of Cry3Bb1 expression levels of MON 88017 are within range of MON 863 Cry3Bb1 protein levels for forage, root and grain and concludes that the differences in expression levels between MON 863 and MON 88017 for leaf, pollen, silk and stover are within the range of biological variability expected for plants grown in different years and locations. As shown by a study attached to the dossier (Dudin 2001) the expression values of Cry3Bb1 in MON88017 were much lower in pollen, root and grain than those of MON 863. However, to obtain direct comparability of the two GM maize lines they should have been grown at similar sites and in the same year in order to achieve comparable environmental conditions. Therefore it cannot be concluded that the two GM maize lines are comparable.	The Panel agrees with the comment. Nevertheless, the comparison with MON863 has not been taken into account for the safety evaluation of MON 88017, which has been assessed based on the data provided for the expression of this event.
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	In the assessment of phenotypic stability two generations have been observed for which the chi-square analysis shows a significant difference between the observed and the expected segregation frequencies (LH198BC1F1 and LH198BC0F1xLH59). The notifier attributed this difference of LH198BC0F1xLH59 to gamete selection caused by glyphosate application to plants of previous generation. However, these differences should be explored in depth and explained further.	The data provided by the applicant is considered sufficient to prove genetic and phenotypic stability over generations. Evidence of gamete selection is provided in scientific literature (Walker et al., 2006).
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.01 Comparative assessment	The maize plants used in the compositional analysis were treated with a single application of Roundup (page 98, technical dossier). For the assessment of herbicide tolerant plants GM plants exposed and not exposed to the herbicide should be included in order to assess whether the expected agricultural condition influences the expression (EFSA	The GMO Panel requested additional information regarding field trials with plants untreated with the herbicide. The additional information provided by the applicant included compositional data on maize MON88017 and comparator lines grown in Northern and Southern Europe, of which the outcomes are also

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			(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). The results of the compositional analyses show significant differences in some of the analytes even across locations. In the US trials significant differences in grain for three analytes (linoleic acid, arachnidic acid, and vitamin B1) were found, for the Argentinian sites 14 significant differences were detected. However, the notifier classifies those differences as "unlikely to be biologically meaningful". However, these differences should gain more attention to clarify the underlying cause as the assessment of compositional equivalence between the GM and the non-GM plant by itself is not considered to be a risk assessment but rather the starting point for further assessments of a GM plant (Codex Alimentarius Commission (2002) Draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. At step 8 of the elaboration procedure.).	summarized in the opinion. In general, these additional data do not give rise to safety concerns over MON88017 maize. See section 4.1.2 of the opinion
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.04 Agronomic traits	The assessed parameters "insect, disease and abiotic stressors" were evaluated qualitatively. Although the notifier differentiated between the different insect species, diseases and abiotic stress factors, a merely qualitative assessment can only indicate rough differences in the susceptibility of a plant to a certain stressor but not subtle differences. Furthermore, the numerical ratings of the ecological interaction characteristics were evaluated across sites. Such a calculation across sites masks differences at single sites due to regionally different frequencies of pest or pathogen infestations. Qualitative differences in ecological parameters of the trials in 2002 were observed (corn rootworm, anthracnose, and chemical injury). The notifier argued that they are likely to be an artefact of the assessment method, not necessarily a biologically meaningful result. However, these differences should trigger more detailed analyses. Especially, the slightly higher corn rootworm incidence for the test substance, the GM maize, in comparison to the control, should be evaluated in more detail (see table 14). Also the data on significantly different seedling vigour across sites in both trial years (2001, 2002) seems to be contradictory. In 2001 seedling vigour across sites was rated 3,7 for MON 88017 and 4,1 for the control and test seedlings	The scope of the application is for food and feed uses, import, processing and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation. See section 5.1.1.1 of the scientific opinion *"The GMO Panel considers also that the small difference in seedling vigour and time of flowering are unlikely to affect the overall fitness and weed potential of the GM maize. There were no other across-site differences in any of the other phenotypic characteristics of the plant tested. The field data do not provide evidence of changes in invasiveness, enhanced weediness or fitness of maize MON88017 plants, except in the presence of glyphosate and of specific target organisms. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON88017 and any change in survival capacity, including over-wintering."*

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			(MON 88017) were considered to be more vigorous than the control (p 70 and table 11 on p 71). In contrast, in the field trial ratings of 2002 (Table 13, p 75), seedling vigour was rated 7,6 for MON 88017 and 6,5 for the control. For this trial seedling vigour for MON 88017 was also rated greater than the control (p 73).	
dAustria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.08 Toxicology	part II The publication from Séralini and co-workers is an interesting contribution in the discussion on risk assessment of genetically modified plants. It highlights the specific significance of careful statistical evaluation of experimental data and could be used as a stimulus for discussing adequate criteria for the evaluation of such type of studies. Regarding the sensible area of risk assessment of genetically modified plants, it should be mandatory to use adequate methods. Regarding both studies, some flaws become obvious. The full assessment of all results requires, that studies give full insight into the experimental design including dietary conditions. This includes not only a full description of the nutrient levels, it should also include data on potentially interfering factors such as pesticide residues or mycotoxin levels. Although the original study contains some statements on dietary adequacy and the absence of interfering substances, no data are presented. This limits the value of the study. Without those data, it is not possible to assess the impact of one single dietary modification on physiological traits in animals. Feed intake is a very important factor when growth is compared. The original study presents in figure 2 some curves on feed intake, however it is hard to draw some valid conclusions from these data. The biochemical parameters show obviously a high degree of variability in the data and I would recommend that over-interpretation should be avoided. It is not uncommon that biochemical parameters are different between dietary achievements and I would recommend to be cautious relating biochemical data to actual disturbances of liver or kidney functions without further parameters. In that case it should also be considered, that blood sampling would be an important factor causing stress and might have some impact on feed intake and body weight development. As consequence of the paper the experimental design, the presentation of data related to the diets and the feed intake and the statistical evaluation methods have to be considered	The 90 days feeding study in rats and the 42-day feeding study in broilers meet Good Laboraory Practice standards for monitoring of interfering factors. After having assessed these studies the Panel concluded that maize MON88017 is as safe as conventional maize varieties and that the overall allergenicity of the whole plant is not changed. Maize MON88017 and derived products are unlikely to have any adverse effect on human and animal health in the context of the intended uses. Moreover it is noted that the Seralini paper pertains to a statistical re-analysis of a 90-day rat feeding study with MON863 maize, hence not with MON88017, on which the EFSA GMO Panel has already published a statement. The comment regarding suitability of animal feeding studies does not address a particular risk for MON88017. The choice as to whether or not a 90-days study should be performed or not has also been addressed in the recently published report of the EFSA GMO Panel working group on animal feeding trials. According to internationally harmonized guidelines for safety assessment of GM foods, animal studies have to be chosen for on a case-by-case basis, which will particularly depend on the outcomes of the comparative assessment between a GMO and a conventional counterpart with a history of safe use.

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			more critically in the future. Another important point could be the duration of feeding trials. The 90 day feeding trial might be too short for a full assessment."	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.08 Toxicology	On page 18, part I of the dossier, the notifier argues concerning the toxicological safety evaluation as follows: "An assessment of safety of the MON 88017 Cry3Bb1 leads to the following conclusions, which are similar to the conclusions reached for the MON 863 Cry3Bb1 protein that was considered as safe by EFSA…". In this respect it has to be stated that EFSA lauched recently a scientific debate on this topic due to new scientific findings. The comment of Prof. Dr. Jürgen Zentek (University of Berlin and University of Veterinary Medicine Vienna), which has been also forwarded to the EFSA on "New Analysis of a Rat Feeding Study with a Genetically Modified Maize Reveals Signs of Hepatorenal Toxicity" from Gilles-Eric Séralini and co-workers, published in Arch. Environ. Contam. Toxicol. (2007), DOI: 10.1007/s00244-006-0149-5 should also be considered in this context: "The group from the Committee for Independent information and Research on Genetic Engineering CRIIGEN, Paris, France, has performed a re-analysis of a feeding study in rats that was originally published by B. Hammond and co-workers in a collaborative paper from Monsanto Company and Covance Laboratories (Food and Chemical Toxicology 44 (2006) 147–160). Both studies come to significantly different conclusions about the biological effects of YieldGard corn (MON 863) grains. The reanalysis confirms the descriptive statistics included in the original paper. However, it indicates, that the maize product has affected weight gain in rats when other statistical models were applied. According to the data, males were growing less than the controls from week 2, and the females more. Additionally, the data on blood biochemistry were interpreted differently compared to the original study. Some of the "liver parameters" and "kidney parameters" at least "appeared to be specifically linked to the GMO diet". Additionally, some effects were described for the urinary excretion of phosphorus and sodium in male animals. The authors summarise in conclusion, that "the two main organs of detoxification, liver and kidney, have been disturbed in this study" and that the	The GMO Panel is aware that the Cry3Bb1 protein in MON863 and in MON 8817 are different. And the evaluation of the Cry 3Bb1 protein in MON 8817 has been performed independently and not with regard to the evaluation of MON863. The Panel considers that the 90 day feeding study has been performed according to the OECD guidelines and no other longer study is required. As also noted above for the previous comment quoting the study on MON863, it is noted that the Seralini paper pertains to a statistical re-analysis of a 90-day rat feeding study with MON863 maize, hence not with MON88017, on which the EFSA GMO Panel has already published a statement. The comment regarding suitability of animal feeding studies does not address a particular risk for MON88017. The choice as to whether or not a 90-days study should be performed or not has also been addressed in the recently published report of the EFSA GMO Panel working group on animal feeding trials. According to internationally harmonized guidelines for safety assessment of GM foods, animal studies have to be chosen for on a case-by-case basis, which will particularly depend on the outcomes of the comparative assessment between a GMO and a conventional counterpart with a history of safe use.

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			original statistical methods were not detailed enough to see disruptions in biochemical parameters. part 1	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.08 Toxicology	The difference in the sequence of the Cry3Bb1 protein compared to the native Cry3Bb1 protein, and the Cry3Bb1 protein in MON 863 is not taken into account specifically. Furthermore the assessment of other potentially expressed polypeptides due to modifications upon construction of MON 88017 is only assessed in silico. To conclude the biological insignificance of the sequence changes in the integrated Cry3Bb1 protein as well as the genetic modifications at the border regions of the insert experimental data should be supplied. Furthermore the notifier bases his safety assessment of MON 88017 maize on the results of a 13 week repeated dose feeding study with rats and a 42 day feeding study with broiler chicken. The dossier states that the 42 day broiler chicken study is the method of choice for evaluating nutritional equivalence. However the additional assumption that the method "is considered a highly appropriate model for confirming the safety of genetically modified maize lines" (page 140, technical dossier), needs to be questioned with regard to conclusions made for subchronic or chronic toxicity. Indeed the study must be considered as a feed conversion study rather than a toxicological study. For safety considerations toxicological endpoints must be assessed rather than performance and meat quality parameters as done in the broiler chicken study supplied. The feeding study with chicken broilers is therefore not appropriate to assess the toxicological safety of MON 88017 maize. The respective conclusion by the notifier has to be substantiated by other experimental results. It remains to be demonstrated by the notifier whether the results 13 week rat study are sufficiently addressing all relevant toxicological endpoints – therefore clarification is needed. In both studies a few significant differences have occurred between MON88017 and the maize variety with a comparable genetic background used as a control (like differences in food consumption and neutrophil lymphocyte cell counts in the female rats fed with a diet consisting of 33% transgenic material). The notifier concludes these	The GMO Panel is aware that the Cry3Bb1 protein in MON 863 and in MON88017 are different, and the evaluation of the Cry 3Bb1 protein in MON 88017 has been performed independently and not with regard to the evaluation of MON863 Cry3 Bb1 protein. 42 day nutritional study is not a tox. study and has only been evaluated as such. The Panel is of the opinion that this study is well performed and accepts its value as nutritional study. The Panel has also considered the outcomes of the 90-days rat feeding study with MON88017 maize. For the change in neutrophil counts mentioned by the member state, it is noted that the relative counts did not differ and that the absolute counts fell within the background range of variation of animals fed with reference lines of maize.

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			differences as not biologically meaningful. However to back this conclusion further evaluation of the differences observed is necessary.	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.08 Toxicology	The description of the toxicological and allergological safety of MON 88017 maize is based on the presumed substantial equivalence and on arguments such as the history of safe use of the respective proteins Cry3Bb1 and CP4 EPSPS and other transgenic maize varieties expressing these proteins, the absence of homology with known protein toxins and allergens, the rapid digestion of the isolated proteins in simulated gastrointestinal fluids etc. (Spök A., Hofer H., Lehner P., Valenta R., Stirn S. & H. Gaugitsch (2005). Risk Assessment of GMO Products in the European Union. Umweltbundesamt Wien, Band 253.)have shown that these assumptions in combination with tests using isolated gene products do not guarantee the absence of toxicity or allergenicity of a product. Therefore little significance can be attributed to the acute toxicological tests with the isolated gene products.	This comment is general and addresses the safety assessment approach. The internationally harmonized approach towards safety assessment of GM foods, as laid down in guidance by Codex alimentarius and EFSA, is based on the initial comparison of a GMO with its conventional counterpart with a history of safe use. Based upon the differences thus identified, it can be further decided which tests are needed to conclude the risk assessment. The assessment of potential allergenicity GM foods usually entails the weight-of-evidence approach recommended by Codex alimentarius. The whole data package pertaining to the safety of MON88017 and its transgenic components has been considered by the Panel. The reference that the member state refers to does not provide new experimental evidence but refers to a critical review of the safety assessment criteria for GM foods.
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.09 Allergenicity	As regards possible interactions between the transgenic proteins the notifier does not expect interactions for reasons of a different mode of action and different site of biological activity of the proteins and unavailable information on a possible mechanism of interaction between these proteins leading to adverse health effects. However, the notifier does not supply specific data that would corroborate the assumed conclusions. With regard to allergenicity the safety of the introduced proteins is justified by sequence comparisons with known allergens, the rapid degradation of Cry3Bb1 and CP4 EPSPS proteins under in vitro conditions, and a low level of expression in MON 88017 leading to a low prevalence of the proteins in foodstuffs. The simplifying presentation of this assumption is not scientifically verified (page 153, technical dossier). There is scientific evidence that such considerations cannot prove the allergological safety of proteins (see references in Spök et al. 2005).	No safety concerns regarding health of consumers have been identified for each of these proteins and no accounts are known of potential interaction between these proteins either, given that their modes of biochemical action of EPSPS and Cry3Bb1 are also different. With regard to the assessment of potential allergenicity of GMOs, it has already been mentioned in response to the previous comment that this usually follows the weight-of-evidence approach recommended by Codex alimentarius. A summary of the issues considered by the Panel is provided in the opinion, including the source of the transgenic proteins, the bioinformatics-supported comparisons of the transgenic proteins to known allergenic proteins, and the resistance towards pepsin during incubation in “simulated gastric fluid”

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Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 07.09 Allergenicity	The description of the toxicological and allergological safety of MON 88017 maize is based on the presumed substantial equivalence and on arguments such as the history of safe use of the respective proteins Cry3Bb1 and CP4 EPSPS and other transgenic maize varieties expressing these proteins, the absence of homology with known protein toxins and allergens, the rapid digestion of the isolated proteins in simulated gastrointestinal fluids etc. (Spök A., Hofer H., Lehner P., Valenta R., Stirn S. & H. Gaugitsch (2005). Risk Assessment of GMO Products in the European Union. Umweltbundesamt Wien, Band 253.)have shown that these assumptions in combination with tests using isolated gene products do not guarantee the absence of toxicity or allergenicity of a product. Therefore little significance can be attributed to the acute toxicological tests with the isolated gene products.	This comment is general and addresses the safety assessment approach. The internationally harmonized approach towards safety assessment of GM foods, as laid down in guidance by Codex alimentarius and EFSA, is based on the initial comparison of a GMO with its conventional counterpart with a history of safe use. Based upon the differences thus identified, it can be further decided which tests are needed to conclude the risk assessment. The assessment of potential allergenicity GM foods usually entails the weight-of-evidence approach recommended by Codex alimentarius. The whole data package pertaining to the safety of MON88017 and its transgenic components has been considered by the Panel. The reference that the member state refers to does not provide new experimental evidence but refers to a critical review of the safety assessment criteria for GM foods.
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 10.05 Interactions of the GM plant with non-target organisms	Toxicity of the Cry3Bb1 toxin to non-target organisms The classification of the target species cannot be considered satisfactory. Weeds must be considered as the primary target organisms of the herbicide tolerant trait introduced into a GM plant and must therefore be considered within the framework of the environmental risk assessment according to Directive 2001/18. The notifier cited a study in order to demonstrate the specificity of the Cry3Bb1 protein to insect species (Head et al. 2001). However, this study is a compilation of several Monsanto-internal studies which are not available from the reference list. These studies should be added to the notification. The notifier argues that a number of diverse insect species has been screened for sensitivity to Cry3Bb1 protein and only the beetles of the chrysomelid family were found to be sensitive as shown by the respective study. However, in this study the sensitivity of the species was classified either “significant mortality” or “no significant mortality” In fact, species that experienced lower mortality (less than 25%) or sub lethal effects (such as growth retardation) were not considered as being “sensitive” to the Cry3Bb1 toxin. Only one other species of Coleoptera which has not a pest status (ladybird beetle) was evaluated for its sensitivity to the Cry3Bb1 toxin. Additionally, the protein used in MON88017 is not equivalent to the protein used in the toxicity study of Head et al. (2001). While the former differs in	See section 5.1.1.4 of the scientific opinion *“The GMO Panel assessed therefore whether the mCRY3Bb1 protein might potentially affect non-target organisms by entering the environment e.g. in manure and faeces from the gastrointestinal tracts of animals fed on maize MON88017”*…. “ *exposure of soil and water environments to CRY toxins of maize MON88017 from disposal of animal wastes or accidental spillage of maize kernels is likely to be very low and localized. Thus exposure of potentially sensitive non-target organisms to the mCRY3Bb1 protein is likely to be very low and of no biological relevance”.* The GMO Panel agrees that the study (Head et al. 2001) provides a compilation of several Monsanto-internal studies. However more detailed studies to demonstrate the specificity of the Cry3bb1 protein to insect species are not considered relevant considering the scope of this application, excluding cultivation (see section 5.2.1.4 of the scientific opinion).

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			six amino acids from the wild-type Cry3Bb1 protein, the latter used the Cry3Bb1 variant 11231 which differs from wild type Cry3Bb1 by four amino acids. The equivalence of these two protein variants with respect to their efficiency towards the intended target species as well as non-target species should have been evaluated. As one of the references added to the notification (Slaney et al. 1992) clearly shows the differential susceptibility of two different species of the same family (Chrysomelidae) to the same Cry-protein, more non-target species of the family should be evaluated for their sensitivity towards the respective protein used in MON 88017.	
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 12.02 Case-specific GM plant monitoring	Case-specific Monitoring The notifier states that accidental spillage of grain may occur during import, handling, storage and processing of maize (p 159, technical dossier). However, the notifier has missed to establish surveillance or management systems which are suitable to monitor and detect possible unintended environmental exposure by accidental spillage or release of MON 88017 nor has the notifer shown that measures are taken that ensure that the reporting of unintended environmental release will be carried out by the relevant stakeholders involved. Therefore, in order to cover the risk of accidental spillage of GM maize MON 88017 a case-specific monitoring plan should be proposed, especially in the light of the apparently greater seedling vigour of MON 88107 compared to the control as shown in the agronomic analysis.	See section 5.2.2 of the scientific opinion "*No specific environmental impact of this GM maize was indicated by the risk assessment and thus no case specific monitoring is required".* *"The GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize MON88017 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003".*
Austria	Ministry of Health, Family and Youth, Dep. IV/9	D, 12.03 General Surveillance of the impact of the GM plant	General surveillance The notifier proposes to submit a surveillance plan similar to the plan submitted for the surveillance of the import and use of NK603 (according to notification C/ES/00/01) and suggests that this plan could serve as a model for MON 88017. The general surveillance report of NK603 maize contained mainly unconfirmed information on maize imports into the different member states within the European Union and a worldwide overview of the approval status of NK603 maize. It also stated that information on NK603 was spread to stakeholders, operators and users and that this was mainly done by the way of press releases, Monsanto's internet website and undefined "other communications". The notifier referred also to the information posted by the European Commission and the EFSA. However, this information is considered to be too general and too imprecise for a surveillance plan of unintended	The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. See section 5.2.2 of the scientific opinion: *"The GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of MON88017 maize since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects".*

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			effects on human or animal health and the environment. Furthermore the notifier stated that networks of associations of traders and storers that handle unprocessed maize grain were provided with specific information for NK603. It remained unclear which specific networks were informed within the individual member states and how it was ensured that the information would reach the relevant stakeholders. It is also unclear why no veterinarians or medical associations were provided with information and included as a surveillance network of NK603 as this maize is mainly used for animal feed and therefore identification of occurrence of adverse effects of the GMO on animal health must be included in the surveillance plan. It is also unclear what the specific technical and safety information consisted of, especially with regard to the "technical fact sheets regarding NK603" which were provided by Monsanto. A copy of this information should have been included. This is also valid for the "options to design processes to collect and guide surveillance information from these networks to consent holders" which were presented to representatives from relevant networks. Also the emergency contact number (hotline) in Spain for Spanish and Portuguese markets cannot be regarded as sufficient for all member states within the European Union. The "standardised adverse effect reporting form" used by Monsanto for those two markets is regarded insufficient in order to detect adverse effects from handling or the use of NK603 maize. Therefore it is crucial that the notifier of MON 88017 definitely improves the general surveillance plan in comparison with the plan of NK603 and that details are provided on the information networks used and the evidence that these surveillance networks actually collect the relevant information and that they have agreed to make any information available on general surveillance of the product. This should be done specifically for the Austrian market before commercialization of GM maize MON 88017.	See section 5.2 of the PMEM opinion (EFSA, 2006b): *"Details of the specific plants 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 commercialization sites, including the type of dialogue that would be established with risk managers in each Member States. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…)".*
Belgium	Belgian Biosafety Advisory Council	A. General information	The modified maize has been presented as more resistant to glyphosate. What's the level of this resistance? Because the modified maize is presented as more resistant to glyphosate, toxicity studies have to be realized to determine the residues level of glyphosate in MON88017, indeed more glyphosate would be applied on MON88017 that on normal	ENV WG See section 5.2.1 of the scientific opinion *"The scope of the application excludes cultivation; therefore concerns regarding the use of glyphosate herbicides on maize MON88017 apply only to imported and processed maize products that may have been*

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			maize. In this dossier, MON 88017 was often declared to be safe as the genes inserted are the same as the one of two other GMOs but some controversies has emerged about the safety of one of these (MON 863). As MON 88017 would 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 theratogenic effects as well as 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 88017 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. Scientists do not consider similar things as equal so that Monsanto can not assume that MON 88017 is safe because similar to wild type maize.	*treated with these glyphosate herbicides in the countries of origin. However, the regulation and risk assessment of glyphosate is within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market (EC, 1991).* FF WG There is substantial amount of literature data showing lack of transfer of transgenic DNA from the consumed plant material to the internal organs of tested animals.Mazza et al 2005, Rossi et al. 2005 The comment regarding the testing for teratogenicity and the comparison between GMOs and their controls are of a more general nature, and do not appear to address a specific issue for MON88017. No indications have been found that would support the notion of potential teratogenicity of MON88017 or of its transgenic constituents. The situations in which such additional animal toxicity experiments would be required have been considered in a recently published report of an EFSA GMO Panel Working Group on Animal Feeding Trials. In general terms, the safety assessment strategy as described in the guidance by the GMO Panel extends upon the internationally harmonized approach for GM food safety assessment as laid down in guidelines of Codex alimentarius. The outcomes of the comparative assessment of MON88017 maize (compared to its conventional counterpart with a history of safe use) have been summarized and concluded upon in the Panel's opinion. The data provided have not given rise to concerns for consumers' health.
Belgium	Belgian Biosafety Advisory Council	C. Information relating to the genetic modification	Methods used for genetic modification, vector and inserted DNA fragments are well described.	The GMO Panel has considered the comment.
Belgium	Belgian Biosafety Advisory Council	D, 01 Description of the trait(s) and characteristics which have	The traits introduced are well known and correctly described.	The GMO Panel has considered the comment.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
		been introduced…		
Belgium	Belgian Biosafety Advisory Council	D, 02 Information on the sequences actually inserted or deleted	The number of insert integrations was evaluated by ScaI restriction of genomic DNA and hybridisation on southern blots. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data support the conclusion that this GMP contains a single integration site of the insert. Of course additional integrations of very small fragments of the insert cannot be excluded. The number of copies inserted at this insertion site was evaluated through XbaI restriction, southern blotting and hybridisation. The probe consisted in a mixture of DNA fragments spanning the entire length of the insert. The data provided in the dossier support the conclusion that a single copy of the insert is integrated. Mendelian segregation of the traits confirms that a single copy of the insert is present and that it is integrated into nuclear DNA. Insert structure and intactness (both expression cassettes) were checked by hybridisation on southern blots of XhoI1 and/or HindIII restricted genomic DNA. Different probes covering the different parts of both expression cassettes were used. The data provided in the dossier support that MON88017 maize contains the expected full-length insert. Absence of integration of vector backbone was checked through hybridisation on Southern blot. The blot was hybridised with a mixture of two probes spanning the entire length of the vector backbone. No integration of such vector sequence was detected. Of course integrations of very small fragments of the vector cannot be excluded. In conclusion the data provided in the dossier support the following claims: - MON88017 maize contains a single integration site of the DNA construct - MON88017 maize contains a single copy of the DNA construct - This insert in MON88017 maize is full length and show the expected structure - No vector backbone is present in the genome of MON88017 maize. Structure and intactness of insert was confirmed by PCR amplification of overlapping DNA fragments spanning the entire length of the insert. In addition these PCR fragments were cloned and sequenced. Compilation of sequences yielded the expected full-length	The comment was considered by the GMO Panel.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			sequence. Sequencing was extended into neighbouring natural plant genomic DNA. A sequence of 878 bp was obtained upstream of the 5' side of the insert. A sequence of 1000 bp flanking the insert on its 3' side was obtained. These sequences corresponded to maize genomic DNA. PCR primers were designed in these flanking regions. They were used in PCR on genomic DNA from non-genetically modified maize. This yielded a 260 bp fragment. Sequencing data of this fragment suggests that integration of the insert was accompanied with limited modifications of the insertion site, i.e. a deletion of 25-27 bp and an addition of 20 bp. It is well known that T-DNA integration often induce this type of modifications.	
Belgium	Belgian Biosafety Advisory Council	D, 03 Information on the expression of the insert	1. Expression of the insert was evaluated through quantitative assays of the two protein products (Cry3Bb1 and CP4 EPSPS). This was done by ELISA on proteins extracted from whole plants or from specific plant organs. Plant material was collected at different growth stages at 3 locations in USA during the 2002 growing season. Additional plant material was harvested in Argentina during the 2003-2004 growing season. The results show that the Cry3Bb1 protein is expressed at different levels in all tested plant parts (leaf, pollen, silk, forage, forage root, grain, stover). The CPA EPSPS protein was also expressed in these plant parts (not tested in silk and stover). Such results were expected as constitutive promoters were used in the expression cassettes. In addition possible expression of fusion proteins was considered. All possible reading frames at insert – genomic DNA junctions on both DNA strands were analysed. All possible peptides were FASTA aligned to different databases. No known immunological epitope was found. 2. SNPs and Microarray method exist to evaluate modification of gene expression. These new technologies which are much more accurate must be introduced in the Panel of tests used to determine the eventual effects of a GMO in tissue.	1. The comment was considered by the GMO Panel. 2. All new technological advances which might add value to the risk assessment process and which are fully validated will continue to be considered by the GMO Panel. (See EFSA Guidance Document, Section II, point 6)

Application EFSA-GMO-CZ-2005-27 (Maize MON88017) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Belgium	Belgian Biosafety Advisory Council	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	1. Genetic stability of the insert was checked by southern analysis of XbaI-restricted genomic DNA. The blots were hybridised with a mixture of 4 DNA fragments spanning the entire length of the insert. This analysis was done over several generations (up to 7). The expected restriction fragments were always observed, suggesting that the insert was stably transmitted from generation to generation. 2. SNPs and Microarray method exist to evaluate modification of gene expression. These new technologies which are much more accurate must be introduced in the Panel of test used to determine the eventual effects of a GMO in tissue.	1. The comment was considered by the Panel. 2. All new technological advances which might add value to the risk assessment process and which are fully validated will continue to be considered by the GMO Panel. (See EFSA Guidance Document, Section II, point 6)
Belgium	Belgian Biosafety Advisory Council	D, 07.01 Comparative assessment	Differences in maize composition statistically significant can not be justified by a “in the range of historical values” this is not a scientific method, values should always be confronted with the control of the same trial.	The Panel considers historical ranges as supportive information after having considered thoroughly the respective relevant control data
Belgium	Belgian Biosafety Advisory Council	D, 07.03 Selection of compounds for analysis	Cry3Bb1 and CP4 EPSPS proteins used for the analysis of the allergenic effects, were produced by E. coli. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004). Freese, W., Schubert, D. 2004. Safety testing and regulation of genetically engineered foods. In Harding, S.E. (Ed.) Biotechnology and Genetic Engineering Reviews 21; 299-324.	GMO Panel therefore accepts the *E. coli* derived Cry3Bb1 and CP4 EPSPS proteins as an appropriate substitute test material for the plant CP4 EPSPS protein in the safety studies. A summary of the studies that have probed the equivalence between plant- and microbially expressed transgenic proteins has been provided in section 5.1.3.1 of the opinion.
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	References for comments under D, 07.08.1 English L., Slatin S L (1992) Mode of action of delta-endotoxins from Bacillus thuringiensis: a comparison with other bacterial toxins. Insect. Biochem. Molec. Biol., 22 (1), 1-7. Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fucsh, R.L. & Padgette, S.R. (1996). The expressed protein in glyphosate-tolerant soybean, 5-enolypyruvylshikimate-3-phosphate synthase from Agrobacterium sp. Strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126(3), 728-740. Hofmann C. et al (1988). Specificity of Bacillus thuringiensis delta-endotoxins is correlated with the presence of high-affinity binding sites in the brush border membrane of target insect midguts. Proc. Natl. Acad. Sci.	EFSA GMO Panel is aware of these references.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			USA, 85, 7844-7848. Van Rie J. et al (1989) Specificity of Bacillus thuringiensis delta-endotoxins. Importance of specific receptors on the brush border membrane of the mid-gut of target insects. Eur. J. Biochem., 186, 239-247. Van Rie J. et al. (1990) Receptors on the Brush border memebrance on the insect midgut as determinants of the specificity of Bacillus thurigiensis delta-endotoxins. Applied and Environmental Microbiology, 56 (5), 1378-1385.	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	D.7.8.4 Testing of the whole GM food/feed 1. 90-days feeding study in rats with MON 88017 maize grain. The objective of these studies was to compare the responses of rats fed MON 88017 grain with the responses of rats fed the conventional control LH59 x LH198 that has background genetics similar to that of the MON 88017 grain (Kirkpatrick, 2005a), and compared with the responses of rats fed 6 commercial reference maize hybrids (Kirckpatrick, 2005b). All maize was grown in the same location at the same time (commercial reference hybrids on different fields). It is not mentioned in this study if MON 88017 maize was grown under glyphosate conditions. The study design included groups of Sprague-Dawley rats (20 rats/sex/group). One group was administered a diet containing 11% (w/w) MON 88017 supplemented with 22% (w/w) control grain. A second group was administered a diet containing 33% MON 88017. A third group was administered a diet containing 33% control grain. Another 6 groups were administered diets containing 33% reference maize varieties. All animals survived, there were no test substance-related clinical observations. Body weights, food consumption and clinical pathology parameters were unaffected by the administration of MON 88017. No test-related effects were found on organ weights, and under macroscopic and microscopic examination. The few difference that were observed (higher mean food consumption and higher absolute neutrophil count in the 33% MON88017 females compared with the control group) fell within the range of responses of the six different groups fed conventional reference varieties of maize grain. 2. Poultry broilers feeding study with MON 88017 maize grain (42 days). The study was undertaken to compare the wholesomeness of MON 88017 grain (treated with glyphosate herbicide? not mentioned in this study) to conventional control (LH59 x LH198) as well as to five	Thank you for this summary which is in agreement with the EFSA GMO Panel opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			commercial reference maize hybrids when fed to rapidly growing Ross x Ross 508 broilers (Taylor et al., 2005). Broilers were fed a starter diet (d0-21) and grower/finisher diet (d21-42) containing appr. 55% and 60% w/w maize, respectively, for all treatments. Treatments were randomly assigned to pens with five blocks for 16 pens (8 male, 8 female) with 10 broilers/pen for a total of 80 pens and 820 broilers. Broilers were weighed by pen on d0 and d42 and individually at study termination. Pen feed intake was determined at d42. At study termination, all surviving birds were processed to determine carcass yield and meat composition. Fat pad measurements were taken for each bird. One broiler/pen was randomly selected and sampled for breast and thigh meat quality assays. Significant diet-by-gender interactions ($p<0.05$) were noted for live weight, final live body weight, chill weight, and thigh weight. No differences were observed in the percentage of moisture, protein, and fat in thigh meat and breast meat of broilers. Comparison of the MON 88017 fed birds to the population of the other diets fed showed no differences on all performance parameters, carcass yields, or meat quality parameters measured. In conclusion, the results of the broiler feeding study showed that there were no biologically significant differences on the parameters tested between broilers fed MON 88017 or the broilers fed control maize. Minor differences noted were consistent with literature values and within natural variability. In conclusion (and as concluded by the applicant), these studies confirm the absence of any toxic effects associated to the introduced proteins and the absence of any unanticipated or pleiotropic effects linked to the genetic modification. In conclusion, there was no evidence of any adverse effects on human or animal health.	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	D.7.8.3 Information on natural food and feed constituents Compositional studies were conducted to establish the nutritional adequacy of MON 88017 maize compared with a conventional control maize with similar genetic background, as well as with other commercially available maize hybrids. A reduction in approx. 23% in vitamin B1 levels was observed in MON 88017 grain samples compared with the conventional control maize (Vitamin B1 was consistently lower at each of the field sites). However, the levels were well within the 99% tolerance interval and well within the	Thank you for this summary which is in agreement with the EFSA GMO Panel opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			literature and historical range for maize grain. Other minor differences in fatty acid or amino acid constituents were not indicative of an overall pattern of change that could be attributed to the modification. In conclusion, no particular natural constituents of maize are considered to be of significant concern to require additional information or further risk assessment.	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	1. Toxicity tests reported in this dossier where done by Monsanto laboratories, what about independent labs toxicity results? 2. The potential for toxicity of CP4 EPSPS and Cry3Bb1 proteins expressed in MON 88017 maize grain may be small, based on the low amount of CP4 EPSPS and Cry3Bb1 proteins found in maize grain, the absence of demonstrated acute toxicity to CP4 EPSPS and Cry3Bb1 in mice at doses greater than the range associated with proteins, the lack of sequence homology between known toxins and the CP4 EPSPS and Cry3Bb1 proteins. 3. It is well-known that the pesticides are endocrinal disruptors. Monsanto reported in this dossier that broilers fed with MON88017 have higher growth index which might be explained by a modification of endocrine axis. In clinical investigations, endocrine measures are considered routine measures in assessing patient health. In this dossier there are no mentions of any endocrine tests! Endocrine axis are the first to be disrupted in illness so that they can not be removed from a toxicity study. The toxicology effects are assumed to be negligible as the new OGM is constituted of the same inserted genes as MON 863 and NK603. In France, “la commission du genie biomoleculaire” has some doubts about the harmlessness of MON863 as there are significant differences in the pathology observed in rats after 90 days of alimentation with MON863. Moreover, the authors indicate that “the Cry3Bb1 proteins produced in MON 88017 and MON 863 share an amino acid sequence identity of 99.8%, differing by only one of 653 amino acids. The single difference occurs at position 166. In MON 88017 and in the wild-type Cry3Bb1 protein, there is an aspartic acid at position 166. In MON 863, there is a glycine instead of an aspartic acid at this position. The physicochemical characterization and functional activity of the Cry3Bb1 protein produced in MON 88017 are equivalent to those of	The GMO Panel is aware that the Cry3Bb1 protein in MON 863 and in MON88017 are different. And the evaluation of the Cry 3Bb1 protein in MON88017 has been performed independently and not with regard to the evaluation of MON863. The Cry3Bb1 and CP4 EPSPS proteins expressed in maize MON88017 showed no homology to known protein toxins and allergens. These proteins were rapidly degraded with simulated mammalian gastric fluid. A subchronic (90-day) feeding study revealed no indications of adverse effects in rats fed diets containing grains from maize MON88017. In addition, a feeding study in broiler chickens provided evidence of nutritional equivalence of maize MON88017 to conventional maize.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			the Cry3Bb1 protein». Two protein even if similar are not equal so it might be that they have the same effects but the contrary is true as well. No assumption of the toxicity can be done on the bases of a similar protein. . In conclusion, longer and more accurate toxicity studies are required to assess the harmlessness of this GMO.	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	D.7.8.1 Safety assessment of newly expressed proteins Monsanto based is safety assessment on comparison with existing toxins but if Cry3Bb1 is not similar to any toxin known this does not mean that it is not toxic! Similar proteins to the two proteins present in MON 88017 maize have been assessed previously for safety (MON 863, NK603). Additionally, a battery of tests designed to evaluate the Cry3Bb1 variant protein and the native CP4 EPSPS protein present in MON 88017 maize for characteristics associated with food allergens and toxins raised no concern. The mature CP4 EPSPS in MON 88017 is identical to the bacterial enzyme of 455 amino acids and is targeted to the plant chloroplast. The Cry3Bb1 in MON 88017 differs from the native Cry3Bb1 by 6 amino acid changes, and differs from the in MON 863 variant by only 1 amino acid. Both novel proteins are expressed at relatively low levels in MON 88017. CryBb1 1. No adverse effects were observed when Cry3Bb1 protein was ingested by mice at a dose of 1930 mg/kg bw. Bioinformatic studies confirmed the absence of any significant amino acid similarity with known toxins and allergens. In vitro digestibility studies demonstrated that the Cry Bb1 variant was rapidly degraded in simulated gastric fluid. Furthermore, the Cry Bb1 variant is not glycosylated in maize. Processing involving heat treatment rendered the CryBb1 variant protein non-functional. The CryBb1 variant protein used in the studies was obtained in an E. coli production system. The equivalency of the MON 88017 maize produced protein to the E. coli- produced protein was evaluated by comparing the molecular weight, immunological reactivity, insecticidal activity and glycosylation. Both proteins were found to be equivalent. 2. The protein is rapidly and completely digested in simulated gastric fluid (SGF). The protein is digested in simulated intestinal fluid (SIF) with formation of fragments being active toxins (technical dossier	See response above.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			pg 120 + fig 24). This seems to be part of its mode of action (English and Slatin (1992); Hofmann et al. (1988); Van Rie et al. (1989, 1990). These toxins bind to specific receptors on the brush border of the gut epithelium of rootworm larvae. Question: Are there studies available which identify these receptors. If so, are these receptors also present in mammals? • Acute oral toxicity (mouse) CP4 EPSPS 1. In previous assessments (e.g. NK603), a battery of tests designed to evaluate the CP4 EPSPS protein for characteristics associated with food allergens and toxins raised no concern. The CP4 EPSPS protein shared no sequence homology with known toxins. There is a rapid digestion of the CP4 EPSPS protein in simulated digestive conditions, susceptibility to heating, and lack of acute toxicity for the CP4 EPSPS protein as determined by the mouse acute oral toxicity study. The CP4 EPSPS protein used in these studies was obtained in an E. coli production system. The equivalency of the MON 88017 maize produced protein to the E. coli- produced protein was evaluated by comparing the molecular weight, immunological reactivity, glycosylation and functional activity. Both proteins were found to be equivalent. 2. • The protein is rapidly and completely digested in SGF. • Digestion in SIF seems to be much slower (Harrison et al. (1996)). • Remark: we disagree with the statement on pg 124 of the technical dossier, which says "… if any of the CP4 EPSPS protein did survive the gastric system, it would be rapidly degraded in the intestine". According to Harrison et al. (1996) 93-95% of added CP4 EPSPS was still present after a 10-min incubation in SIF. CP4 EPSPS activity had decreased to < 9% of the initial level after incubation of 285 min! • Acute oral toxicity (mouse)	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	D.7.8.4 Testing the whole GM food/feed Chronic toxicity has been demonstrated for MON 863. Subchronic study demonstrated that there is a significant increase in neutrophil count in some groups of females which is not justified in a scientific manner. Confrontation of data with data of other studies is not valid. There is a lack of a longer chronic study in other to assess effects of long term ingestion of MON88017.	For haematology parameters a statistically significantly higher absolute neutrophil count was observed only in females fed 33% MON88017 maize. There were, however, no differences in the relative neutrophil counts between rats fed 33% MON88017 maize and the concurrent controls. The mean absolute neutrophil counts observed in female rats fed 33% MON88017 maize were within the range in background variation. The serum chemistry parameters, urine analysis and

Country	Organisation	Reference	Comment	EFSA GMO Panel response
				microscopic examinations showed no effects related to feeding rats with diets containing 11% or 33% MON88017 maize.
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	3. Monsanto claims no allergenicity for the new proteins because they don't share aminoacids sequences with known allergens but again these proteins are new in human alimentation and so there is a need of specific scientific studies. References for D, 7.9 Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C, Sampson HA. 2003 : Clinical and laboratory investigation of allergy to genetically modified foods. Environ Health Perspect. 2003 Jun;111(8):1114-21. Chowdhury, E.H., Kuribara, H., Hino, A., Sultana, P., Mikami O.,, Shimada N.,, Guruge, K.S., Saito, M., Nakajima, Y. 2003. Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. J. Anim. Sci. 81: 2546-2551. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. 2005 : Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. Cytometry B Clin Cytom. 2005 Mar;64(1):28-33. Ebo DG, Stevens WJ. 2001 : IgE-mediated food allergy--extensive review of the literature. Acta Clin Belg. 2001 Jul-Aug;56(4):234-47. Helm RM. 2003 : Food biotechnology: is this good or bad? Implications to allergic diseases. Ann Allergy Asthma Immunol. 2003 Jun;90(6 Suppl 3):90-8.	The EFSA GMO Panel has considered the "weight of evidence" regarding potential allergenicity of MON88017 and its transgenic proteins, in line with its guidance and the internationally harmonized approach as described in Codex alimentarius guidelines. This weight of evidence also includes, besides the outcomes of the bioinformatics-supported comparisons, the history of allergenicity, if any, of the sources of the transgenic proteins and the in-vitro resistance of the transgenic proteins towards proteolytic enzymes.
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	1. Maize itself (Zea mais) rarely induces allergic reactions in man as a food nor as a pollination plant The new proteins Cry3Bb1 and CP4 EPSPS were already evaluated for allergenicity in the context of MON 863 and NK603 maize. The risk for allergenicity can be assessed by combining different approaches (Helm 2003): - content of the protein(s) in the food/feed - digestibility of the protein(s) and stability in acid proteases in the food/feed - comparison of the amino acid structure of the protein(s) with known allergens - testing with specific IgE from allergic patients - testing in animal	The allergenicity assessment can be found in section 5.1.5 where it is stated that: 'Bioinformatics-supported comparisons of the amino acid sequence of the plant-expressed Cry3Bb1 and CP4 EPSPS proteins with sequences of known allergens were performed. No peptides showing relevant overall identity to an allergen sequence were identified for Cry3Bb1 and CP4 EPSPS proteins using the FASTA search algorithm using allergen database (AD8). In addition, when the criterion of an identical 8-aa contiguous amino acid

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			models For three of these parameters the proteins Cry3Bb1 and CP4 EPSPS showed a good profile: - low content of proteins Cry3Bb1 and CP4 EPSPS in the maize end product - good digestibility in acid peptic digestion It has to be mentioned nevertheless that not all allergens are stable proteins (eg Mal d 1 from apple) (Ebo et al. 2005) As far as the comparison of the proteins Cry3Bb1 and CP4 EPSPS with known allergen structures is concerned: - protein Cry3Bb1 showed some similarity with the Anisakis simplex tropomyosin Ani s3. The overlap of 120 aa contained four gaps and showed 27.5 % identity with an E score of 1.1. The longest stretch of continuous aa was 3; this was considered as non significant. Follow up of this situation is advised since tropomyosin are to be considered as pan-allergen in a high number of living animal, with possible cross reactivity (Ebo and Stevens 2001). - protein CP4 EPSPS had an alignment of 30.5 % identity with Dermatophagoides farinae Der f 2over 82 aa with a high E score of 0.41. The longest stretch of contiguous aa was 5. This similarity was evaluated as insignificant. Follow up of this situation is advised since Dermatophagoides sp belong to the most frequently occurring inhalation allergens in moderate climate zones such as in important parts of the US and Europe. No reports in medical databases were found on allergenicity of the proteins Cry3Bb1 and CP4 EPSPS. Continuous surveillance is advised. It has also to be taken in consideration that other forms of allergic reactions than IgE mediated are possible (Bernstein et al. 2003) 2. MON 88017 maize contains 2 new proteins with distinct properties. The toxic and allergenic effects of both proteins were individually discussed. The applicant believes that the general surveillance plan endorsed by EFSA for NK603 can also serve as a model for MON 88017. However, it is not sufficiently stated that there is no synergism between both proteins with regard to possible detrimental effects. On P.109, Part I of the Technical Dossier, it is stated that these proteins are similar to the proteins expressed in MON 863 and NK603, respectively, that have been considered safe by EFSA. This is not in agreement with the draft report of the EFSA (2006) “Safety and Nutritional Assessment of GM Plant derived Foods/Feed The role of animal feeding trials”, where it is emphasized that a safety assessment of a novel food/feed should be based	stretch was applied, the Cry3Bb1 and CP4 EPSPS sequences yielded no positive outcomes. The studies on degradation of Cry3Bb1 and CP4 EPSPS proteins with simulated mammalian gastric fluid, which are also relevant for the assessment of potential allergenicity, have been described in Section 5.1.3.2. The studies showed that most of the test proteins were degraded by pepsin within seconds. Based on the information available the EFSA GMO Panel considers that the newly expressed Cry3Bb1 and CP4 EPSPS proteins are unlikely to be allergenic.’

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			on a case by case approach. Obviously, this is not the case in this dossier. Furthermore, Monsanto has not done any effort to isolate sufficient Cry3Bb1 and CP4 EPSPS proteins from MON 88017 maize, but they used Cry3Bb1 and CP4 EPSPS proteins produced by E. coli (P.116, Part I of the Technical Dossier). It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese & Schubert, 2004). Monsanto used simulated gastric and intestinal fluids to test the digestion of Cry3Bb1 and CP4 EPSPS proteins. It has been shown that a rapid in vivo degradation of Cry proteins (Cry1Ab) does not always occur (Chowdhury et al., 2003). Furthermore, Spök et al (2005) have shown that digestibility studies can not be considered suitable tools to address the allergenic potential of a protein.	
Belgium	Belgian Biosafety Advisory Council	D, 07.10 Nutritional assessment of GM food/feed	There are no indications suggesting nutritional inconveniences in comparison to conventional maize varieties. It was concluded from the animal performance in broiler studies that there was a nutritional equivalence compared with conventional control lines (Taylor et al., 2005). The applicant only discusses MON 88017 in this dossier. What effects can be expected if this novel food/feed is used in diets containing other GM food/feed, such as soy beans, rape seed, rice, …? The effect of a combined use of MON 88017 with other novel foods/feeds in diets for animals and humans is not extensively investigated. Are interactions between proteins from MON 88017 and proteins from other GM plants excluded?	This issue is not specific for MON88017 proteins and proteins from other GM plants and should be addressed on a case by case basis.
Belgium	Belgian Biosafety Advisory Council	D, 08 Post-market monitoring of GM food/feed	As no long term toxicity studies have been done, we can not exclude long term effect of GMO consumption. That's why a follow-up of the GM food is required post-market.	The Panel is of the opinion that no further long term studies are necessary.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Belgium	Belgian Biosafety Advisory Council	D, 10.03 Potential for gene transfer	The probability that (spillage + establishment + contamination) is limited at some parts of the itinerary (e g at ports), but not necessarily along the transportation routes. Even though it can not survive the winter, maize from spilled seeds can develop one generation on the sites of spilling, leading to potential dissemination of pollen. 1% of the pollen beyond 50 m (Sears and Stanley-Horn, 2000) does not seem negligible to me. If we do not know the routes, we do not know if maize is grown along the roads We feel that more specific details are needed regarding the packing and other means of confinement during transportation and storage Sears M.K. & Stanley-Horn D., 2000 : Impact of Bt corn pollen on monarch butterfly populations . 6th Int. Symposium on the Biosafety of GMOs, p. 120-130.	The GMO Panel considers that more specific details on packing and other means of confinement are not needed considering that maize is highly domesticated and generally unable to survive in the environment without cultivation.
Belgium	Belgian Biosafety Advisory Council	D, 10.03 Potential for gene transfer	The probability that (spillage + establishment + contamination) is limited at some parts of the itinerary (e g at ports), but not necessarily along the transportation routes. Even though it can not survive the winter, maize from spilled seeds can develop one generation on the sites of spilling, leading to potential dissemination of pollen. 1% of the pollen beyond 50 m (Sears and Stanley-Horn, 2000) does not seem negligible to me. If we do not know the routes, we do not know if maize is grown along the roads I feel that more specific details are needed regarding the packing and other means of confinement during transportation and storage	See answer section D.10.03
Belgium	Belgian Biosafety Advisory Council	D, 10.06 Effects on human health	Monsanto should provide more accurate toxicity studies in order to demonstrate its hypothesis of no human toxicity.	This point has been addressed in the opinion and sufficient data have been supplied by the applicant.
Belgium	Belgian Biosafety Advisory Council	D, 10.07 Effects on animal health	Studies of Taylor et al. (2005) indicated that broiler mortality based on diets containing MON 88017 fell within the range reported for commercial maize varieties. See also comment on D.7.9.	See answer to comment D.7.9
France	MINEFI - DGCCRF	D, 02 Information on the sequences actually inserted or deleted	Information on the sequences actually inserted or deleted Le séquençage d'un fragment d'ADN de 7126 pb appartenant au génome du maïs a été réalisée, comportant l'insert et les régions flanquantes en 5' (878 pb) et 3' (1000 pb). L'étude du site d'insertion aux extrémités 5' et 3' dans le maïs MON 88017 et le maïs conventionnel montre qu'au cours du processus d'intégration de l'ADN-T, se sont produites d'une	The Panel asked for more information on the preinsertion locus. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical,

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			part, une délétion de 25-27 pb et d'autre part, une insertion additionnelle de 20 pb dans le génome de MON 88017 ; Afin de s'assurer qu'aucune nouvelle séquence n'a été créée par l'insertion, une étude bioinformatique complète a été réalisée pour rechercher la présence d'ORF (open reading frame) putatives dans les 6 cadres de lecture au niveau des régions de bordures de l'insert. La comparaison des séquences déduites de ces ORF putatives, pouvant générer un peptide de plus de 8 acides aminés, avec des séquences figurant dans des banques d'allergènes, de toxines, de motifs peptidiques n'a pas mis en évidence d'homologie significative entre ces peptides putatifs et des séquences connues répertoriées dans ces banques de données. Cependant aucune information n'est fournie pour savoir si l'intégration de l'évènement MON 88017 s'est faite dans une région fonctionnelle ou non du génome du maïs. L'Afssa estime qu'il conviendrait de réaliser une analyse par northern blot sur des extraits d'ARN totaux provenant de différents tissus de la plante (feuilles, tiges, racines et graines) pour savoir si l'intégration de l'évènement MON 88017 s'est faite dans une région fonctionnelle ou non du génome du maïs.	and compositional analyses showed that MON88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and that the insertion of the transgene *per se* does not pose a safety hazard.
France	MINEFI - DGCCRF	D, 07.10 Nutritional assessment of GM food/feed	Nutritional assessment of GM food/feed Une étude d'alimentarité a été réalisée chez des poulets (350 mâles et 350 femelles, 10 répétitions par traitement et par sexe) nourris pendant 42 jours avec deux régimes [correspondant aux périodes de démarrage (0-21 jours), de croissance et de finition 21-42 jours)] à base de maïs MON 88017 (54 et 59 %) en comparaison avec des poulets nourris dans les mêmes conditions avec du maïs témoin ayant le même fonds génétique et 5 variétés commerciales de maïs cultivées aux Etats-Unis ; L'équivalence de composition chimique entre le maïs MON 88017 et les maïs témoins et les teneurs en mycotoxines des rations ont été vérifiées et que le dosage de la protéine Cry3Bb1 montre sa présence dans les rations à base de maïs MON 88017 et son absence (non détectée) dans les maïs témoins. Aucune information n'est cependant fournie sur la présence de la protéine CP4 EPSPS dans la ration alimentaire. Les observations ont porté sur 8 paramètres zootechniques, 7 données de découpe et 3x2 données de composition des muscles et que le taux de mortalité enregistré (0,9 %) au cours de l'expérimentation est non lié au traitement ; Les résultats,	The applicant has provided a 42-day feeding study with broiler chicken to analyse the nutritional value of grain from the MON88017 maize treated with glyphosate, the near isogenic control (LH59xLH198) and five commercial corn varieties. Out of 56 statistical comparisons performed between the test and the control animals, there were statistically significant differences in feed intake of males, average thigh weight of males and percent drum weight of chill weight of males. These statistically significant differences are in the biological range of such studies and are not considered as biologically meaningful. The outcomes of the broiler feeding study support the conclusion on the compositional analysis summarized above that grains of maize MON88017 are compositionally and therefore nutritionally comparable to grains of the non-GM comparator and commercial maize lines.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			après analyse statistique, montrent qu'on observe : - aucune différence due aux traitements entre les animaux nourris avec le maïs MON 88017 et le maïs témoin ou les variétés commerciales testées pour ce qui concerne les performances pondérales, la consommation d'aliment, l'efficacité alimentaire, le taux de survie des oiseaux ; - aucune différence, à l'issue de l'expérience, en ce qui concerne les données relatives aux caractéristiques de la carcasse (rendement à l'abattage, qualité de la viande) et que le poids moyen des reins ainsi que le gras abdominal ne sont pas modifiés. Sur la base de l'analyse de ces résultats, on peut conclure à une équivalence nutritionnelle du maïs grain MON 88017 avec son témoin non génétiquement modifié. L'Afssa estime qu'il conviendrait de disposer des dosages de la protéine CP4 EPSPS dans la ration alimentaire des poulets. De plus, l'Afssa souligne le fait que la pertinence des études chez le rat et chez le poulet aurait été renforcée si ces études avaient été réalisées avec du maïs MON 88017 traité par du glyphosate. Automatic tranlsation Nutritional assessment off GM food/feed a study d' alimentarity was carried out in chickens (350 males and 350 females, 10 repetitions by treatment and sex) nourished during 42 days with two modes [corresponding to the completion 21-42 and growth, launching periods (0-21 days) days)] containing corn MY 88017 (54 and 59%) in comparison with chickens nourished under the same conditions with pilot corn having same the funds genetics and 5 commercial varieties of corn cultivated in the United States; L' equivalence of chemical composition between pilot corn MY 88017 and corn and the contents of mycotoxins of the rations were checked and that the proportioning of the Cry3Bb1 protein shows its presence in the rations containing corn MY 88017 and its absence (not detected) in pilot corn. No information n' is however provided on the presence of protein CP4 EPSPS in the food intake. The observations related to 8 zootechnical parameters, 7 data of cutting and 3x2 given composition of the muscles and that the death rate recorded (0,9%) during l' experimentation is nonrelated to the treatment; The results, after statistical analysis, show qu' one observes: - no difference due to the treatments between	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			the animals nourished with corn MY 88017 and pilot corn or the commercial varieties tested concerning the ponderal performances, the food consumption, the food effectiveness, the rate of survival of the birds; - no difference, at the conclusion of the experiment, with regard to the relative data with the characteristics of the carcass (output to demolition, quality of the meat) and that the average weight of the kidneys as well as the abdominal fat is not modified. On the basis of analysis of these results, one can conclude with a nutritional equivalence from the corn grain MY 88017 with his witness not genetically modified. L' Afssa estimates qu' it would be advisable to have proportionings of protein CP4 EPSPS in the food intake of chickens. Moreover, l' Afssa underlines the fact that the relevance of the studies in the rat and chicken would have been reinforced if these studies had been carried out with corn MY 88017 treaty by glyphosate.	
Germany	Federal Agency for Nature Conservation (BfN)	General comments	Additional comments by the Federal Agency for Nature Conservation: The Federal Agency for Nature Conservation considers that more data are needed to come to a final risk assessment of MON 88017 maize. In particular thoroughly analysed and more detailed data on the phenotypic equivalence and on the expression of the new proteins are required. With respect to this, it is our opinion that the number of field seasons and locations is not adequate for a commercial use of the GMO. The risk assessment of the applicant is not sufficient, because it is based on the assumption of substantial equivalence. We do not share the opinion of the applicant that the data provided prove the substantial equivalence of MON 88017 to the isogenic line. As a consequence of the above mentioned deficits the GMO should not be approved at present.	Having considered the information provided in the application, the GMO Panel requested from the applicant additional data on the composition of maize MON88017 not treated with glyphosate in comparison with conventional control maize. The applicant provided the requested information.The GMO Panel considered the observed compositional differences between maize MON88017 and its non-GM comparators in the light of the field trial design, the biological variation and the levels of the compounds in conventional maize varieties, and concludes that maize MON88017 is compositionally equivalent to the non-GM comparators and conventional maize varieties, except for the introduced trait. The GMO Panel is of the opinion, that the set of compositional data supplied is in compliance with the principles described in the guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a).
Germany	Federal Agency for Nature Conservation (BfN)	A. General information	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/CZ/2005/27.	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Germany	Federal Agency for Nature Conservation (BfN)	A, 07 Where appropriate, the conditions for placing on the market the food(s) or…	Products consisting of maize seed should be accompanied by an instruction leaflet including the information that resulting plants are able to tolerate herbicides containing glyphosate and cannot be managed by using such herbicides.	Outside the remit of the GMO Panel
Germany	Federal Agency for Nature Conservation (BfN)	D, 02 Information on the sequences actually inserted or deleted	(d) The organisation of the inserted genetic material at the insertion site including se-quence data of the inserted material and of the flanking 5' and 3' region According to applicants' information 878 bp of maize genomic DNA flanking the 5' end of the insert and 1000 bp of maize genomic DNA flanking the 3' end of the insert that show homo¬logy to maize DNA are reported. However, a complete bioinformatical analysis of the ob-tained sequences was not performed and should be requested. In this respect, a bioinfor-matical analysis of the 5'- and the 3'-flanking regions of the insert to assess the presence of genetic regulatory elements (such as potential promoter and polyadenylation sequences) is missing and should be asked for from the applicant. Minor Comment: In figure 17 and figure 18 (see technical dossier part I) the results of the overlapping PCR analyses demonstrating the linkage of the individual genetic elements within the insert in MON 88017 are demonstrated. For the sake of completeness, for product A and B (see Fig. 17) and product F and G (see Fig. 18), respectively, PCR using plasmid PV-ZMIR39 DNA as a template should be performed as a negative control.	The Panel asked for more information on the preinsertion locus. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical, and compositional analyses showed that MON88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and the insertion of the transgene *per se* does not pose a safety hazard.
Germany	Federal Agency for Nature Conservation (BfN)	D, 03 Information on the expression of the insert	(b) Parts of the plant where the insert is expressed The applicant states that the range of MON 88017 Cry3Bb1 protein levels (µg/g fwt) in MON 88017 was within the range of MON 863 Cry3Bb1 protein levels in MON 863 for for-age, forage root, and grain. However, Dudin et al. (2001) reported for the Cry3Bb1 protein in grain of MON 863 a concentration of 70 µg/g fwt (range 49-86) whereas Bhakta et al. (2003) reported a concentration of 13 µg/g fwt (range 8.7-19) in grain of MON 88017. Explanation of the difference between the data of Bhakta and Dudin and proper statistical tests should be given to establish the equivalence of MON 88017 and MON 863 protein levels. Bhakta, N.S., Hartmann, A.J. and Jennings, J.C. (2003) Cry3Bb1 and CP4 EPSPS protein levels in corn tissues collected from MON 88017 corn produced in U.S. field trials conducted in 2002. Monsanto	The comparison with MON863 has not been taken into account for the safety evaluation of MON 88017, which has been assessed based on the data provided for the expression of this event.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			Technical Report, MSL 18823 and Individual data Bhakta 2003. Dudin, Y.A., Tonnu, B., Albee, L.D. and Lirette, R.P. (2001) Amended report for MSL16559: B.t. Cry3Bb1.11098 and NPTII protein levels in samples tissue collected from corn event MON 863 grown in 1999 field trials. Monsanto Technical Report, MSL 17181.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 03 Information on the expression of the insert	Additional comments by the Federal Agency for Nature Conservation: The expression is not well characterized. The analysis of expression levels in MON 88017 maize is limited to three field sites during the 2002 field season in the U.S.A (Bhakta et al., 2003). Only for grain there are additional data of four field sites during the 2002/2003 field season in Argentina (Dudin et al., 2005). Since 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 expression levels and cannot be regarded as sufficient for a market release. The limited data set does not allow to test for unintended adverse effects on the expression due to gene-environment interactions as different climatic and environmental conditions. Bhakta, N.S., Hartmann, A.J. and Jennings, J.C. (2003) Cry3Bb1 and CP4 EPSPS protein levels in corn tissues collected from MON 88017 corn produced in U.S. field trials conducted in 2002. Monsanto Technical Report, MSL 18823 and Individual data Bhakta 2003. Dudin, Y.A. and Jennings, J.C. (2005) Assessment of CP4 EPSPS and Cry3Bb1 protein levels in corn grain from MON 88017 produced in 2003-2004 Argentina field trials. Monsanto Technical Report, MSL 19781 and Indi-vidual data Dudin and Jennings 2005.	The GMO Panel considers the information provided to be sufficient on the basis that the scope of the application covers only food, feed, import and processing
Germany	Federal Agency for Nature Conservation (BfN)	D, 04 Information on how the GM plant differs from the recipient plant in: …	Additional comments by the Federal Agency for Nature Conservation: With regard to a final assessment further information is required. Information including pa-rameters, methods and proper statistical tests should be given to establish the phenotypic and ecological equivalence of MON 88017 with conventional maize (isolines) and maize with the two single traits. Unexpected adverse effects should be given special attention in the risk assessment of GMO`s, therefore results from field trials prior to market release play an im-portant role. The data provided to show the agronomic equivalence of MON 88017 (Rosenbaum et al., 2003; Pester and Woddrum, 2003) are not sufficient. The size of the plot	The scope of the application is for food and feed uses, import, processing and does not include cultivation. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation. Considering the scope of the application, excluding cultivation, the GMO Panel considers that the data provided to show agronomic equivalence of MON88017 maize are sufficient.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			(two rows , 5-6 m in length) and the parameters observed allow only a very limited assess-ment of potential change in ecological traits. Pester, T.A. and Woodrum, C.L. (2003) Phenotypic and ecological observations of MON 88017 corn in U.S. field trials during 2002 for an assessment of equivalence and weed potential. Monsanto Technical Report, MSL 18944 and Individual data Pester 2003. Rosenbaum, E.W., Wilste, C.C. and Horak, M.J. (2003) Phenotypic and ecological observations of MON 88017 corn in 2001 U.S. field trials for an assessment of equivalence and weed potential. Monsanto Technical Re-port, MSL 17652 and Individual data Rosenbaum et al. 2003.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.01 Comparative assessment | D, 07.03 Selection of compounds for analysis	Additional comments by the Federal Agency for Nature Conservation: Similar to the analysis of Bt-expression levels, the compositional analysis relies solely on the 2002 U.S.A. and 2003/04 Argentinean field trials. The comparative assessment of MON 88017 maize is limited to three field sites during the 2002 field season in U.S.A. (McCann 2003) and four field sites during the 2002/2003 field season in Argentina (McCann et al., 2005). Since nutrients 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 nutrient levels and cannot be regarded as sufficient for a market release. The limited data set does not allow to test for unintended adverse effects due to different climatic and envi-ronmental conditions. Additional data should be collected to account for a minimum of three growing seasons and six locations. McCann, M.C. (2003) Evaluation of the composition of forage and grain collected from MON 88017 and MON 88017 x MON 810 corn grown in 2002 U.S. field trials. Monsanto Technical Report, MSL 18556. Part I – Technical dossier 202. McCann, M.C., Trujillo, W.A. and Sorbet, R. (2005) Evaluation of the composition of corn forage and grain col-lected from MON 88017 grown in 2003-2004 Argentina field trials. Monsanto Technical Report, MSL 19365.	Extensive compositional data in line with OECD recommendations have been provided for maize grown in USA and Argentina, which are considered representative of regions exporting maize and derived products to the EU and in line with the requirements set out in the EFSA Guidance. Additional compositional data have been provided by the applicant in response to a request by the Panel for such data (in relation to herbicide treatment of the tested crops). Also these supplementary data corroborated the findings for the two field trials' data already provided, which do not raise safety concerns. For "gene x environment" interactions analysis as suggested by the member state, a much more extensive testing scheme would be required (also more extensive than suggested by the member state). It is noted that field trial design and statistical analysis are considered by a dedicated working group of the EFSA GMO Panel, for which a draft opinion was recently issued for consultation.
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.01 Comparative assessment | D, 07.03 Selection of compounds for	According to applicants¡¦ information the compositional analysis was conducted according to the OECD consensus document on compositional consideration for new varieties of maize (OECD, 2002). However, the comparative assessment included only compositional analysis of forage and grain, although the cited OECD consensus document specifies	The GMO Panel considered the observed compositional differences between maize MON88017 and its non-GM comparators in the light of the field trial design, the biological variation and the levels of the compounds in conventional maize varieties, and concludes that maize MON88017 is compositionally equivalent to the non-GM

Country	Organisation	Reference	Comment	EFSA GMO Panel response
		analysis	more maize matrices in which nutritional and compositional parameters should be analyzed for human food use and animal feed use, respectively. In this regard, suggested nutritional and compo-sitional parameters to be analysed in the following maize matrices for human food use are: oil („_ fatty acids), starch („_ proximate analysis), grits/meal/flour („_ proximate analysis, amino acids, fatty acids), and kernels („_ proximate analysis, minerals, vitamins, amino ac-ids, fatty acids, phytic acid, raffinose, furfural, ferulic acid, p-coumaric acid). Furthermore, suggested nutritional and compositional parameters to be analysed in the following maize matrices for animal feed are: kernel („_ proximate analysis, amino acids, fatty acids, calcium, phosphorus, phytic acid) and silage („_ proximate analysis, calcium, phosphorus). Therefore, the applicant should be requested to provide a compositional analysis containing all maize matrices in which nutritional and compositional parameters should be analyzed for human food use and animal feed use according to the OECD consensus document on compositional consideration for new varieties of maize. At least, the applicant should be asked to explain the choice of the investigated maize tissues and state a reason for the sufficiency of the pre-sented results. OECD. (2002) Consensus document on compositional considerations for new varieties of maize (Zea Mays): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Organization of European Coopera-tion and Development, Series on the Safety of Novel Foods and Feeds, OECD ENV/JM/MONO (2002)25.	comparators and conventional maize varieties, except for the introduced trait.. The GMO Panel is of the opinion, that the set of compositional data supplied is in compliance with the principles described in the guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a).
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.02 Field trials	Production of material for comparative assessment was conducted at three replicated field sites in major maize-growing areas of the U.S.A. during the 2002 field season as well as at four replicated field sites across Argentina during the 2003-2004 field seasons. The applicant stated that all test plots received an application of Roundup herbicide according to label in-struction. This procedure does not come up to the EFSA Guidance Document (2004) which recommends: “In the case of herbicide tolerant GM plants, it is advisable to include both blocks of genetically modified plants exposed to the intended herbicide and blocks not ex-posed to the herbicide. This design would allow assessment of whether the expected agricul-tural condition might influence the expression of the studied parameters.” An	The same answer as above

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			explanation by the applicant why the experimental design does not include test plots not exposed to Roundup herbicide should be requested. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of geneti-cally modified plants and derived food and feed, the EFSA Journal (2004) 99, 1-94.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.04 Agronomic traits	Additional comments by the Federal Agency for Nature Conservation: Climatic and environmental conditions of the experiments should be included to show that representative conditions have been covered. The mere statement of the applicant: 'a range of environmental and agronomic conditions representative for a major temperate region for maize production' clearly needs to be more substantiated. With regard to a final assessment further information is required. Information including parameters, methods and proper statis-tical tests should be given to establish the phenotypic and ecological equivalence of MON 88017 with conventional maize and maize with the two single traits. Because unexpected adverse effects should be given special attention in GMO, results from field trials before the market release play an important role in the risk assessment. Additional data should be collected to account for a minimum of three growing seasons and six locations.	Extensive compositional data in line with OECD recommendations have been provided for maize grown in USA and Argentina, which are considered representative of regions exporting maize and derived products to the EU and in line with the requirements set out in the EFSA Guidance. Additional compositional data have been provided by the applicant in response to a request by the Panel for such data (in relation to herbicide treatment of the tested crops). Also these supplementary data corroborated the findings for the two field trials' data already provided, which do not raise safety concerns. For "gene x environment" interactions analysis as suggested by the member state, a much more extensive testing scheme would be required (also more extensive than suggested by the member state). It is noted that field trial design and statistical analysis are considered by a dedicated working group of the EFSA GMO Panel, for which a draft opinion was recently issued for consultation.
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.06 Effect of the production and processing	According to the EFSA Guidance Document (2004) "the applicant should assess any poten-tial risk associated with horizontal gene transfer from the processed product to humans, ani-mals and the environment, should intact and functional DNA remain after the processing events". However, the applicant neither examined whether intact and functional DNA remain actually after the processing events nor he estimated any potential risk associated with hori-zontal gene transfer from the introduced DNA sequences within the processed product. An adequate examination should be provided by the applicant. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of geneti-cally modified plants and derived food and feed, the EFSA Journal (2004) 99, 1-94.	The issue of horizontal gene transfer is part of the environmental risk assessment (ERA), which has to be carried out by the applicant and has been considered by the GMO Panel.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.08 Toxicology	D.7.8.1. Safety assessment of newly expressed proteins Minor Comment: The presented time course studies of digestion of the protein Cry3Bb1 in simulated gastric fluid (see technical dossier part I: Fig. 23, lane 13-25) and in simulated intestinal fluid (see technical dossier part I: Fig. 24, lane 13-25) demonstrated by western blot analysis lack data on the amount of protein loaded per lane. The applicant is requested to amend the missing information. D.7.8.4. Testing of the whole GM food/feed Minor Comments: The applicant might be asked to explain why he included only the test substance (MON 88017 maize grain) and a control substance (LH59 x LH198 maize grain) and not also a reference substance (commercially available laboratory rodent chow) in the study design of the 13-week feeding study in rats. Since the applicant refers to two test diets describing the repeat-dose animal feeding study in broiler chickens (Tayler et al., 2005), he should point out that only one of the two test diets contained maize MON 88017 while the other comprised maize MON 88017 x MON 810. Taylor, M.L., Huyn, Y., Hartnell, G.F., Nemeth, M.A., Karunanandaa, K., George, B. and Glenn, K.C. (2005) Amended report for MSL 19355: sponsor summary of report for study 02-01-50-20 (Comparison of broiler performance when fed diets containing MON 88017, MON 88017 x MON810, control, or commercial corn). Monsanto Technical Report, MSL 19877.	The Panel has considered the details of the pertinent reports (e.g. Bonner, 2003c for in-vitro degradation of Cry3Bb1) and made its own summary in the opinion, focusing also on the comparison of MON88017 and its control in the broiler study
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.08 Toxicology	D.7.8.1. Safety assessment of newly expressed proteins According to the EFSA Guidance Document (2004) it is essential that the tested E. coli pro-duced protein is equivalent to the newly expressed protein as it is expressed in the GM plant, for example by comparison of the amino acid sequence. While the analysis conducted to establish the equivalence of the plant made CP4 EPSPS protein to the E. coli produced CP4 EPSPS protein included at least a N-terminal sequence analysis, no such test was per-formed to demonstrate the equivalence of the Cry3Bb1 protein produced by E. coli and in maize MON 88017. Accordingly, the applicant should be requested to perform also a N-terminal sequence analysis to confirm the equivalence of the Cry3Bb1 expressed in MON 88017 to E. coli produced protein. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of geneti-cally modified plants and derived food and feed, the	As described in the Panel's opinion, various tests have been performed in order to probe the equivalence of the plant- and microbially expressed transgenic Cry3Bb1 proteins, including MALDI-TOF of constituent peptides, SDS PAGE, Western, insect bioassay and glycosylation. It is concluded in the Panel's opinion that the Panel accepts the microbial Cry3Bb1 as test material. Given that CP4 EPSPS, the acute oral toxicity study with CP4 EPSPS has already been evaluated in the frame of previous applications, to which reference is made in the Panel's opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			EFSA Journal (2004) 99, 1-94. Potential structural similarities of the MON 88017 Cry3Bb1 protein to known proteins and toxins were evaluated using the FASTA sequence alignment tool. According to applicants' information the results of the FASTA sequence alignments demonstrate the lack of structur-ally relevant similarities between MON 88017 Cry3Bb1 and toxins or other pharmacologically active proteins that may adversely impact human or animal health. In this regard, we wish to stress the fact that an investigation of the original data (McCoy and Silvanovich, 2003b) could not be performed as the corresponding pdf-file could not be opened via the EFSAnet. McCoy, R.L. and Silvanovich, A. (2003b) Bioinformatics analysis of the Cry3Bb1 protein as expressed in corn event MON88017 utilizing the AD4, TOXIN5, and ALLPEPTIDES databases. Monsanto Technical Report, MSL 18709. An acute oral toxicity assessment was conducted to evaluate the potential toxicity on mice from exposure to E. coli produced CP4 EPSPS protein. However, no raw data are presented in the cited study by Harrison et al. (1996). Only a body weight analysis of whole groups but no individual data is shown. Moreover, it is stated that there were no significant differences in cumulative body weight and food consumption, although no data were shown in the paper which, all in all, provides only vague information. Hence, the applicant should be requested to deliver all row data corresponding to the conducted acute oral toxicity study. Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L. and Padgette, S.R. (1996) The expressed protein in glyphosate-tolerant soybean, 5- enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, is rap-idly digested in vitro and is not toxic to acutely gavaged mice. J.Nutr., 126, 728-740. According to the EFSA Guidance Document (2004) the safety assessment of newly ex-pressed proteins (in this case: CP4 EPSPS and Cry3Bb1) should include amongst others an examination of the stability of the plant expressed protein under processing and storage con-ditions and the expected treatment of the food/feed. In this context, the influences of tem-perature and pH changes should be examined and potential modifications of the proteins (e.g. denaturation) and/or production of stable	

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			protein fragments generated through such treatments should be discussed. As such an analysis is missing in the available application, the applicant should be asked to deliver adequate data in addition. Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of geneti-cally modified plants and derived food and feed, the EFSA Journal (2004) 99, 1-94.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.09 Allergenicity	D.7.9.1. Assessment of allergenicity of the newly expressed protein Minor Comment: The applicant stated that the protein Cry3Bb1 has already been evaluated for allergenicity in the context of MON 863 maize application that received a positive scientific opinion by EFSA. This is true, however, we would like to point out that the Cry3Bb1 protein expressed in MON 863 and the Cry3Bb1 protein expressed in MON 88017 do not share 100 percent se-quence identity.	The Panel acknowledges that this minor change has also been considered in its opinion
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.10 Nutritional assessment of GM food/feed	Additional comments by the Federal Agency for Nature Conservation: Similar to the analysis of Bt-expression levels, the compositional analysis relies solely on the 2002 U.S.A. and 2003/04 Argentinean field trials. The comparative assessment of MON 88017 maize is limited to three field sites during the 2002 field season in U.S.A. (McCann 2003) and four field sites during the 2002/2003 field season in Argentina (McCann et al., 2005). Since nutrients 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 nutrient levels and cannot be regarded as sufficient for a market release. The limited data set does not allow to test for unintended adverse effects due to different climatic and envi-ronmental conditions. Additional data should be collected to account for a minimum of three growing seasons and six locations. McCann, M.C. (2003) Evaluation of the composition of forage and grain collected from MON 88017 and MON 88017 x MON 810 corn grown in 2002 U.S. field trials. Monsanto Technical Report, MSL 18556. Part I – Technical dossier 202. McCann, M.C., Trujillo, W.A. and Sorbet, R. (2005) Evaluation of the composition of corn forage and grain col-lected from MON 88017 grown in 2003-2004 Argentina field trials. Monsanto Technical Report, MSL 19365.	Extensive compositional data in line with OECD recommendations have been provided for maize grown in USA and Argentina, which are considered representative of regions exporting maize and derived products to the EU and in line with the requirements set out in the EFSA Guidance. Additional compositional data have been provided by the applicant in response to a request by the Panel for such data (in relation to herbicide treatment of the tested crops). Also these supplementary data corroborated the findings for the two field trials' data already provided, which do not raise safety concerns. For "gene x environment" interactions analysis as suggested by the member state, a much more extensive testing scheme would be required (also more extensive than suggested by the member state). It is noted that field trial design and statistical analysis are considered by a dedicated working group of the EFSA GMO Panel, for which a draft opinion was recently issued for consultation.

Application EFSA-GMO-CZ-2005-27 (Maize MON88017) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Germany	Federal Agency for Nature Conservation (BfN)	D, 08 Post-market monitoring of GM food/feed	Additional comments by the Federal Agency for Nature Conservation: The data provided to show the human and animal safety of MON 88017 maize on the basis of its substantial equivalence to conventional maize (except for the introduced traits) are not sufficient. Therefore, a post-market monitoring of the use of MON 88017 maize for food and feed is regarded obligatory and a post-market monitoring plan covering this issue is required.	This is a risk management issue. However, as no safety concerns regarding human and animal health have been identified, no specific risks are known to which post-market monitoring could be used to address
Germany	Federal Agency for Nature Conservation (BfN)	D, 10 Potential changes in the interactions of the GM plant with the biotic…	Additional comments by the Federal Agency for Nature Conservation: Water and soil organisms may be exposed to the Cry proteins of MON 88017 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 concentra-tion of Cry3Bb1 in organic waste material, litter or sewage. The possibility of an accumulation of the Cry proteins 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 Cry proteins.	The scope of the application is for food and feed uses, import, processing and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation. See section 5.2.1.1 of the scientific opinion *"The GMO Panel considers also that the small difference in seedling vigour and time of flowering are unlikely to affect the overall fitness and weed potential of the GM maize. There were no other across-site differences in any of the other phenotypic characteristics of the plant tested. The field data do not provide evidence of changes in invasiveness, enhanced weediness or fitness of maize MON88017 plants, except in the presence of glyphosate and of specific target organisms. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON88017 and any change in survival capacity, including over-wintering."*
Germany	Federal Agency for Nature Conservation (BfN)	D, 12 Environmental Monitoring Plan	Additional comments by the Federal Agency for Nature Conservation: The safety of MON 88017 cannot be fully assessed because of the deficiencies of the appli-cation listed under the comments on chapters D.7.1, D7.4 and D.9. More data are needed to come to a final conclusion of the environmental risk assessment of MON 88017 maize. De-pending on the results of an updated environmental risk assessment the conclusions con-cerning the necessity of a case-specific post-market monitoring may need to be revised.	The GMO Panel considers the information provided in the application sufficient to conclude on the environmental risk assessment of the MON88017 maize.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	Additional comments by the Federal Agency for Nature Conservation: As stated by the applicant, the scope of the application of MON 88017 maize is for import, processing and all uses for food and feed. The applicant provides an environmental monitor-ing plan. This post-market monitoring plan does not fully meet the objectives defined in An-nex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC). Therefore, a plan suitable to meet these objectives is requested.	The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. See section 5.2.2 of the scientific opinion "*The GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of MON88017 maize* since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects".
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.02 Case-specific GM plant monitoring	Additional comments by the Federal Agency for Nature Conservation: We do not share the opinion of the applicant that a case-specific monitoring is not necessary. During transport, storage, package or processing incidental spillage of MON 88017 maize can occur. Furthermore, the exposure of MON 88017 maize and the Cry proteins to the envi-ronment during or after the production process (e.g. through organic waste material or sew-age) and during or after animal consumption (e.g. through manure) is given. Therefore, case-specific monitoring has to focus on pathways, how MON 88017 maize can enter the envi-ronment. The applicant is requested to provide a case-specific monitoring plan including in-formation: • how losses and spillage of MON 88017 maize during transport, storage, package, proc-essing and use as feed will be monitored, • how the exposure of organic waste material, sewage or manure containing MON 88017 maize or Cry proteins to the environment during or after the production process or animal consumption will be monitored. If spread, persistence and accumulation of MON 88017 maize and the Cry proteins in the receiving environment occur, further observations of possible impacts on organisms, food chains and habitats in the specific environment are required.	See section 5.2.2 of the scientific opinion "*No specific environmental impact of this GM maize was indicated by the risk assessment and thus no case-specific monitoring is required*". *"The GMO Panel advises that appropriate management systems should be in place to restrict seeds of maize MON88017 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003".*
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	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 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	The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. See section 5.2 of the PMEM opinion (EFSA, 2006b): "*Details of the specific plans and methods of monitoring*

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			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 monitor-ing plan. Submitting monitoring reports on an annual basis is sufficient.	*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 (…)."*
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	Additional comments by the Federal Agency for Nature Conservation: According to Directive 2001/18/EC general surveillance is a compulsory part of the monitor-ing. 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 provided by the applicant is not in line with Annex VII of Direc-tive 2001/18/EC and the supplementing guidance notes (2002/811/EC). The applicant pre-sents a plan in which e.g. observations by external people, monitoring results from existing networks and internet information will be used for general surveillance. The professional qualification of these people and details of the listed sources are not specified. Both parts of the monitoring plan – case-specific monitoring and general surveillance – have to meet the following requirements: A fully specified list of monitoring parameters has to be defined. 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 observa-tions (time and number of visits to collect data) and the monitoring locations including num-ber and size. Furthermore an operating schedule giving full details of points in time is re-quested. If monitoring data are collected by external people or existing networks the monitoring exper-tise of the external people involved in the monitoring activities and detailed information about participating networks (e.g. name, EU country, responsible authority, availability, scope of monitoring,	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. The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. See section 5.2.2. of the scientific opinion *"The GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of MON88017 maize".* See also 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 (…).*

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			composition of the network) have to be specified. Binding agreements/contracts with third parties (external persons and/or existing networks) are requested which clearly determine what data are provided and how these data are made available. 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. The applicant is requested to indicate how the monitoring plan is adapted to different local condi-tions where appropriate. The methods of data analysis including the statistical methods have to be elaborated in full detail. The applicant is requested to state how the condition of the environment before the placing on the market of MON 88017 maize is described (determination of the baseline status of the receiving environment as a point of reference of the monitoring). 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 be-yond the period of the consent. Furthermore, the general surveillance plan has to focus on possible pathways how MON 88017 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 appli-cant is requested to provide an appropriate monitoring plan to observe the spread, persis-tence and accumulation of the Cry proteins in organism and environmental media (soil, air, water).	See section 5.2.2 of the scientific opinion *"No specific environmental impact of this GM maize was indicated by the environmental risk assessment and thus no case-specific monitoring is required".*
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.06 Reporting the results of monitoring	Additional comments by the Federal Agency for Nature Conservation: The applicant is required to report on the results of the monitoring including all issues of case-specific monitoring and general surveillance on an annual basis. All raw data have to be made available if requested. The applicant is requested to state how the results of the monitoring will be published.	The applicant provided a "Monitoring plan for the import and use of GM MON88017 maize in the EU" according to Annex VII of Directive 2001/18/EC. The applicant states in the monitoring plan that they will "*submit general surveillance reports on an annual basis, following placing on the market*".

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Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Greece	Hellenic Food Authority	D, 02 Information on the sequences actually inserted or deleted	During the integration of the insert into the plant genome, a 20 bp deletion occurs. It is not clear if the insert interrupts a functional DNA region. Additionally, there is no reference concerning the chromosome where the MON 88017 inserted.	Further analysis performed by the applicant demonstrate that the insertion locus is a single copy sequence located in the nuclear genome of maize, in chromosome 4. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical, and compositional analyses showed that MON 88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and that the insertion of the transgene *per se* does not pose a safety hazard.
Greece	Hellenic Food Authority	D, 03 Information on the expression of the insert	It is mentioned that no empirical evidence exists to suggest that transcription of DNA sequence at the 5' or 3'junctions of the DNA inserted in MON 88017 occurs. Despite the bioinformatics analysis conducted, a transcriptional analysis should be carried out, in order to exclude the possibility of the existence of a transcript.	The bioinformatics analyses provided by the applicant allow concluding that in the unlikely event that any of the junction sequences were to be transcribed and translated, the translated products would not pose any safety concern.
Malta	Malta Environment and Planning Authority	General comments	The following need some explanation: the raised value of neutrophils in female rats fed the diet containing the 11% and 33% GM maize; the exclusion of data for the levels of expressed protein in the grain.	The Panel has also considered the outcomes of the 90-days rat feeding study with MON88017 maize. For the change in neutrophil counts mentioned by the member state, it is noted that the relative counts did not differ and that the absolute counts fell within the background range of variation of animals fed with reference lines of maize.
Malta	Malta Environment and Planning Authority	A. General information	Do the rat feed studies follow OECD standards?	Yes , OECD guidelines (e.g. 408 for 90-day oral rodent toxicity)

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Malta	Malta Environment and Planning Authority	B, 03 Survivability; (a) ability to form structures for survival or dormancy, …	As the seedling vigour of some of the maize seeds has been altered, would this not be expected to alter the risk of invasiveness? eg acacia seedling vigour determines its survival and invasiveness	The scope of the application is for food and feed uses, import, processing and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation. See section 5.2.1.1 of the scientific opinion *"The GMO Panel considers also that the small difference in seedling vigour and time of flowering are unlikely to affect the overall fitness and weed potential of the GM maize. There were no other across-site differences in any of the other phenotypic characteristics of the plant tested. The field data do not provide evidence of changes in invasiveness, enhanced weediness or fitness of maize MON88017 plants, except in the presence of glyphosate and of specific target organisms. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of maize MON88017 and any change in survival capacity, including over-wintering."*
Malta	Malta Environment and Planning Authority	C. Information relating to the genetic modification	There are reports that for maize Mon810 and maize 863 the integration site for the Agrobacterium generated transfer is concentrated is near a retrotransposon element. As maize Mon88017 is generated in the same way, the same argument may apply. Further molecular biology techniques may be required to rule out presence of retrotransposons. Also the risk assessment should incorporate a monitoring plan that takes into account that the plant genes located close to the insert may undergo alterations.	The GMO Panel is not aware of any data indicating an specific *Agrobacterium*-mediated integration in retrotransposon-related sequences. A large fraction of maize genome is composed of retrotransposons, so it is not suprising that insertion occurs in these elements. However, in this particular event, this is not the case.
Malta	Malta Environment and Planning Authority	C. Information relating to the genetic modification	The expressed protein CryBb1 differs from the native protein expressed in Bacillus thuringiensis by six amino acids. The protein in maize Mon 88017 differs from maize MON863 another modified product from Monsanto by only six nucleotide bases but due to degeneracy in the genetic code only one different amino acid results. Recent studies on MON863 have shown that rats fed on this maize showed abnormal changes, however, these were not reported in the sub-chronic toxicity tests performed on rats fed on Mon88017 maize, which seems rather odd with only one different amino acid from MON863.	The safety of MON88017 CryBb1 protein has been assessed based on the data provided for this event, which was considered sufficient.

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Malta	Malta Environment and Planning Authority	D, 03 Information on the expression of the insert	The Malta Environment and Planning Authority together with its Biosafety Co-ordinating Committee have assumed that the maize in the compositional analysis was not treated with glyphosate, hence there is both endogenous maize epsps activity as well as the bacterial epsps activity introduced by genetic modification. However, application of glyphosate in the field inhibits any endogenous epsps enzyme in the maize plant that is normally involved in the synthesis of aromatic amino acids. Therefore in the field, one would expect lower levels of aromatic amino acids than those recorded in the notification. Is this correct? kindly explain	The compositional analysis data provided in the dossier does not support this hypothesis. It has been shown that with other GM maize events conferring glyphosate tolerance, glyphosate treatment does not influence composition.
Malta	Malta Environment and Planning Authority	D, 03 Information on the expression of the insert	No data for the levels of expressed protein in the grain seems to have been presented	The applicant has presented data on expression levels in the grain (see section 3a of the Technical Dossier)
Malta	Malta Environment and Planning Authority	D, 07.02 Field trials	Could the herbicide treatment/growth conditions for plants subjected to the compositional analysis be specified. In particular the Malta Environment and Planning Authority and the Biosafety Co-ordinating Committee (BCC) would like to know whether the plants were treated with glyphosate or not prior to analysis of the composition of amino acids. This is important as a few are on the border of the range quoted in the literature. Spraying with glyphosate would be expected to reduce endogenous epsps enzyme in the plant leaving the bacterial epsps active.	Yes, the plants have been treated with glyphosate and the Panel also requested from the applicant additional data on the composition of maize MON 88017 not treated with glyphosate in comparison with conventional control maize.
Norway	Directorate for Nature Management	General comments	According to the Norwegian Gene Technology Act, possible contributions to a sustainable development and possible benefits to the society and ethical considerations through the use of a GMO shall be taken into consideration when evaluating a GMO notification. We therefore ask the Notifier to provide more information on possible contributions to sustainable development and benefits to the society. This information should include the expected changes in herbicide use that the cultivation of line MON 88017 will lead to in the countries where this maize line is being cultivated, and possible environmental effects caused by this change in herbicide use. We also would like to have the Notifier´s evaluation of which effects cultivation of line MON 88017 could have on non-target organisms, especially on threatened species of Collembola.	See EFSA guidance document (EFSA, 2006a) "*Socio-economic and ethical issues are also outside the scope of this guidance*". The scope of the application is for food and feed uses, import, processing and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation.

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Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Norway	Directorate for Nature Management	A. General information	In our opinion a more comprehensive and binding post market monitoring plan is needed. The submitted plan describes examples of networks that will be involved and possible representative environments that will be monitored, but fails to point out clearly enough who is responsible for carrying out the monitoring, which methodologies will be used to detect unanticipated effects, and which networks/key stakeholders the Notifier plans to involve in the monitoring activities. It is also unclear how it will be ensured that the people involved will have the necessary expertise to detect unanticipated effects.	The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. See section 5.1.2 of the scientific opinion: *"The GMO Panel is of the opinion that the general approaches and measures of the monitoring plan proposed by the applicant are in line with the EFSA opinion on post-market environmental monitoring (EFSA, 2006b) as well as with the intended uses of MON88017 maize".* See section 5.2 of the PMEM opinion (EFSA, 2006b): *"Details of the specific plants 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 commercialization sites, including the type of dialogue that would be established with risk managers in each Member States. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…)".*
Norway	Directorate for Nature Management	D, 02 Information on the sequences actually inserted or deleted	Analyses of the flanking sequences on each side of the inserted sequence have been carried out in order to detect possible open reading frames that might code for proteins with toxic or allergenic effects. The application lacks, however, an analysis of the possibility that the DNA-sequence has been inserted in an intron or in a regulatory sequence. We therefore ask the Notifier to provide more information on the probability that this might be the case, and also a discussion of which consequences this could have for the overall gene expression in line 88017.	The Panel asked for more information on the preinsertion locus. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical, and compositional analyses showed that MON 88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and that the insertion of the transgene *per se* does not pose a safety hazard.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Norway	Directorate for Nature Management	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 88017 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 mCry3Bb1 protein in food and feed from maize MON 88017 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 mCry3Bb1 must comment upon the mouse studies showing humoral antibody response of Cry1A proteins. Further, although the mCry3Bb1 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 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.	The GMO Panel is of the opinion that the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity caused by maize consumption or contact. Furthermore, maize is not a common allergenic food, and only a rare cause of occupational allergy may occur. The EFSA GMO Panel has considered the “weight of evidence” regarding potential allergenicity of MON88017 and its transgenic proteins, in line with its guidance and the internationally harmonized approach as described in Codex alimentarius guidelines. This weight of evidence also includes, besides the outcomes of the bioinformatics-supported comparisons, the history of allergenicity, if any, of the sources of the transgenic proteins and the in-vitro resistance of the transgenic proteins towards proteolytic enzymes. Also the potential unintended change in intrinsic allergenicity of the host maize has been considered in the opinion

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			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.	
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… | D, 07.08 Toxicology | D, 07.09 Allergenicity | D, 12 Environmental Monitoring Plan	SPANISH COMMENTS EFSA/GMO/CZ/2005/27: MON 88017 MAIZE D.02. Information on the sequences actually inserted or deleted The molecular characterization of the MON 88017 maize is not complete in some aspects. In particular more information is needed on: 1. The complete sequence of the flanking regions of the two inserts. 2. Information about homologies found with flanking regions, in order to know if any gene of the plant could be interrupted. 3. Size and location of the open-reading-frame (ORF) created by inserts. D.07. 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 Digestion studies Regarding in vitro digestion studies of the CP4 EPSPS and Cry3Bb1 proteins in simulated gastric fluid (appendixes Leach et al (2002b) and Boneer (2003c), respectively), notifier should explain which are the biggest polypeptides obtained, which are their molecular weights, and experimental tests carried out. Moreover, the CP4 EPSPS protein used in this study (Leach et al 2002b) was produced from E.coli, so the results obtained could not be reliable. D.07.09-Allergenicity Sequence homology searches The notifier has presented studies to compare the CP4 EPSPS and Cry3Bb1 proteins with allergens and toxins proteins (appendixes McCoy (2003a) and McCoy (2003b), respectively), using 8 amino acid blocks. However, in the FAO/WHO 2001 report it is recommended to use 6 amino acids instead of 8 amino acid blocks. An analysis with 6 amino acids should be performed (in addition to the 8 amino acids assay) to avoid the increase of false negatives. There are examples of different epitopes of highly allergenic	1. The dossier contains data on the sequences flanking the insert (page 55 of the technical Dossier). 878 nt of the 5 'flanking and 1000 nt of the 3' flanking have been sequenced and were subjected to bioinformatic analyses 2. The Panel asked for more information on the preinsertion locus. Data provided as additional information showed that a 26 bp fragment of genomic DNA at the target site was deleted and a 20 bp fragment was inserted. The insert lies 174 bp upstream of a region showing high sequence similarity to ESTs annotated as corresponding to putative purine permeases. Phenotypical, agronomical, and compositional analyses showed that MON 88017 is equivalent to conventional maize, except for the expected trait, indicating that the insertion of the transgene has not altered the expression of an essential gene and that the insertion of the transgene *per se* does not pose a safety hazard. 3. The analysis of the ORFs is done in Section D3(c) of the Technical Dossier. D.07: with regard to the criterion for the minimum length of contiguous identical amino acids in the bioinformatics-supported comparison between the transgenic proteins and allergens, guidance from both EFSA and Codex alimentarius notes that this should be based on a scientific rationale, without fixing a specific value. It is however known from a range of studies in peer-reviewed literature that the six-amino-acid threshold is likely to give

Country	Organisation	Reference	Comment	EFSA GMO Panel response
			proteins of 6 amino acids. There are also recent references in the bibliography (Taylor and Hefle, Food Allergy Assessment for products derived through plant biotechnology, en Biotechnology and Safety Assessment, Academia Press, 2002, 325-345) reaffirming the need of using 6 amino acids when comparing sequences. D.12. Monitoring Plan The consent holder should provide further details of the arrangements of the monitoring plan, in particular for general surveillance, indicating which existing network programs could be used, the type of information that should be collected and a more detailed monitoring methodology in order to have a monitoring plan which could be implemented in a harmonised manner among the importer Member States.	rise to many false positives as compared to the 8-amino-acid threshold. D.12 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 (…).*
The Netherlands	Ministry of Agriculture, Nature and Food Quality and Ministry of Health, Welfare	D, 03 Information on the expression of the insert	The report by Bonner et al. (2003b)in an annex to the dossier describes how immunoblotting with anti-EPSPS antisera not only recognized CP4 EPSPS (45 kDa), as expected, but also two additional bands at 48 kDa and 55 kDa, whose N-terminal sequences could not be aligned with the sequence of the CP4 EPSPS. If molecular characterization indicates that these bands are truly different from CP4 EPSPS and to be linked with the genetic modification, then their safety for human and animal health should be assessed according to the EFSA guidance document.	The applicant has provided data demonstrating that a single protein of the expected Mw and recongnized by the CP4 EPSPS antibody is expressed in MON 88017. The 48 and 55 kDa proteins that co-purify with the plant produced CP4 EPSPS protein are not recognized by the CP4 EPSPS antibody. The Panel sees no reason to believe that these two unrelated proteins would be linked to the genetic modification.
The Netherlands	Ministry of Agriculture, Nature and Food Quality and Ministry of Health, Welfare	D, 12.03 General Surveillance of the impact of the GM plant	The Dutch CA under the 2001/18/EC has the following procedural point: A general surveillance plan is supplied. The applicant makes a distinction between reporting direct and indirect effects in the monitoring plan. According to the applicant direct effects will be reported annually and indirect effects only at the stage of re-evaluation or at the end of a given consent. The Dutch CA under the 2001/18/EC is of the opinion that the applicant should report unexpected direct and indirect effects annually.	The GMO Panel has requested additional information from the applicant in relation to the general surveillance plan. Direct and indirect effects will be reported annually.