Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry for Health Family and Youth	General comments	The notifier repeatedly states that the available information on MON810 does not justify the development of additional information for its reassessment (e.g. Annex 3.3 on molecular characterisation and 3.4 protein expression and compositional analysis). This conclusion however is not justified as evidenced by the multitude of our comments on the notification regarding ambiguities of the submitted information, the lack of information in most sections of the dossier as well as concerns based on recent scientific publications. On the contrary the assessment strategy should have been reviewed with a view to experiences with cultivation of MON810 in Europe, and the study designs adapted to the current state of the art throughout the sections of the dossier and specifically for the ERA. We request that data reflecting updated test strategies be submitted and discussed by the notifier according to the detailed comments below.	Additional information has been requested to the applicant by the molecular characterisation group, food-feed group and environmental group.
Austria	Ministry for Health Family and Youth	General comments	References made in the Austrian Comments: Nguyen H. T. (2004): Sicherheitsforschung und Monitoringmethoden zum Anbau von Bt-Mais: Expression, Nachweis und Wirkung von rekombinantem Cry1Ab in heterologen Expressionssystemen. Diss. Georg-August-Univ. Göttingen Nguyen H.T. & J.A. Jehle (2007): Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. Journal of Plant Diseases and Protection, 114(2): 820-87. Pascher, K. & M. Dolezel (2005). Koexistenz von gentechnisch veränderten, konventionellen und biologisch angebauten Kulturpflanzen in der österreichischen Landwirtschaft. Handlungsempfehlungen aus ökologischer Sicht. BMGF, Forschungsbericht der Sektion IV, Band	(-)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
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Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Austria	Ministry for Health Family and Youth	General comments	References made in the Austrian Comments: Harwood, J.D., Wallin, W.G., Obrycki, J.J. (2005): Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol. Ecol. 14, 2815-2823. Harwood et al. (2007): Temporal detection of Cry1Ab endotoxins in coccinellid predators form fields of Bacillus thuringiensis corn. Bulletin of Entomological Research 97, 643-648. Heissenberger A., Traxler A., Dolezel M., Pascher K., Kuffner M., Miklau M., Gaugitsch H., Kasal V. & S. Loos (2003): Durchführung von Untersuchungen zu einem ökologischen Monitoring von gentechnisch veränderten Organismen. Bundesministerium für Soziale Sicherheit und Generationen, Sektion VII, Forschungsbericht 4/03. Hellmich, R. L.; Siegfried, B. D.; Sears, M. K.; Stanley-Horn, D. E.; Daniels, M. J.; Mattila, H. R.; Spencer, T.; Bidne, K. G. & Lewis, L. C. (2001): Monarch larvae sensitivity to Bacillus thuringiensis-purified	(-)

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
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Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
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Austria	Ministry for Health Family and Youth	General comments	References made in the Austrian Comments: Anderson, P. L.; Hellmich, R. L.; Sears, M. K.; Sumerford, D. V. & Lewis, L. C. (2004): Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. In: Environ. Entomol. 33, pp. 1109-1115. Anderson P.L., Hellmich R.L., Sears M.K., Sumerford D.V., Lewis L.C. (2005): Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. Env. Entomol. 33: 1109-1115. Andow, D.A. & Hilbeck. A. (2004). Science-based risk assessment for nontarget effects of transgenic crops. Bioscience 54: 637-649. Aumaitre, A. 2004. Safety assessment and feeding value for pigs, poultry and ruminants of pest protected (Bt) plants and herbicide tolerant (glyphosate, glufosinate) plants: interpretation of experimental results observed worldwide on GM plants. Italian Journal of Animal Science 3(2):107-121. Birch A. N. E., Griffith B. S., Caul S., Thompson J., Heckmann, L. H., Krogh P. H., Cortet J. (2007): The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: a review of the ECOGEN project. Pedobiologia 51,	(-)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
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Austria	Ministry for Health Family and Youth	D, 02 Information on the sequences actually inserted or deleted	The molecular characterisation is based on a combination of Southern Blot, PCR and Sequencing data with reference to Scanlon et al., 2007. However the included data display technical shortcomings. Specifically the analysis of insert and copy number by Southern Blot of HindIII digested genomic DNA of MON810 hybridised with probes spanning the whole transgenic insert detects 2 fragments instead of	NcoI digestion indicates a single insert and sequence information indicates two HindIII sites in proximity.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			the single fragment which is expected. Additionally both fragments seem to be of higher molecular weight than indicated by the notifier (see Fig. 6 technical dossier p. 36). The appearance of the additional hybridising fragment is explained as a result of partial digestion of the genomic DNA by the notifier. To avoid any ambiguities we request that the notifier submits additional data unequivocally supporting his conclusions. The notifier is furthermore requested to clarify the use of plasmids PV-ZMBK07 and PV-ZMGT10 as controls for the molecular analysis of MON810. As indicated by EFSA in their letter from 24th April several US patents for MON810 refer to another plasmid (pMON15772) concerning generation of the event. In case plasmid pMON15772 was indeed used to establish the MON810 event covered by the present notification the sections C (Information relating to the genetic modification) as well as D.2 (Information on the sequences actually inserted or deleted) have to be completely revised accordingly. Concerning the molecular structure at the insertion site the presented data do not justify the conclusion that the sequences flanking the transgenic insert are native to the maize genome. The necessary data for this demonstration are neither included in the dossier nor the cited reference (Borovkov et al., 2001). We therefore request that the notifier submits adequate data to support his conclusion (i.e. a PCR analysis to demonstrate that the primers 1 and 4, which were generated for amplification of flanking sequences in MON810 (see Fig. 15 technical dossier p.49), also amplify the locus of insertion in unmodified maize DNA). Furthermore we request submission of data to indicate potential changes of the native maize sequences at the site of insertion due to the	It has been clarified with the applicant that PV-ZMBK07 and pMON15772 are identical plasmids. Monsanto is currently evaluating the incorrect information in the patent application. Additional information provided in 2007 confirmed the DNA sequences of the 5' and 3' DNA flanking regions originally provided but supplied additional sequence information. This revealed an additional 400 bp of maize DNA at the 3’ flank and an additional 1000 bp of maize DNA at the 5’ flank. Updated analysis of ORFs indicated no hypothetical chimeric proteins and no homologies with potential toxins or allergens, confirming the original bioinformatic assessment. However, the updated bioinformatic analyses did reveal that one ORF, previously identified as sharing homology with the importin protein, shared a higher level of identity to a more recently sequenced

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			genetic modification. Publicly available information showed evidence that the integration of the MON810 insert has probably caused a complex recombination event (Hernandez et al., 2003 in Rosario et al., 2008). We request that this is discussed by the notifier. Since the submitted analysis of the sequences of the analysed regions flanking the insert in MON810, does not contain information on the chromosomal location of the insert, we request additional information on the chromosomal location as included in other applications according to Reg. (EC) 1829/2003 (i.e. RFLP data or FISH data).	protein, the HECT-ubiquitin protein. There is phenotypic and compositional equivalence between MON 810 maize and its conventional counterparts so there is no evidence of any safety implications resulting from the interruption of this gene sequence. Information on chromosomal location is not required by EFSA´s Guidance Document.
Austria	Ministry for Health Family and Youth	D, 03 Information on the expression of the insert	The notifier has not presented any new data on expression of GM maize MON810. The values reported in the notification are identical to those reported in the previous notification of MON810 submitted according to Directive 90/220/EEC (C/F/95/12-02). These data were established in field trials conducted in the USA in 1994 and at some European locations in 1995 using MON810 lines other than the lines developed for commercial application in Europe in recent years. Since MON810 has been cultivated in several EU member state countries in recent years, data should be available on expression patterns under different environmental conditions. The notifier has failed to present any of these data. This is particularly relevant in view of recently published data that Cry1Ab expression levels vary considerably between individual plants in MON810 (Nguyen & Jehle, 2007). We therefore request additional data from the notifier assessing the differences in expression between different varieties, years and locations reflecting the recent application of MON810 in Europe and a discussion of results in the light of the	The original data provided was comprehensive and did not indicate any safety concerns with regard to protein expression levels.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			differences found by Nguyen and Jehle (2007). Additionally, information on the developmental expression of the inserted transgenes is missing and should be submitted by the notifier (EFSA 2006, D.3).	
Austria	Ministry for Health Family and Youth	D, 03 Information on the expression of the insert	Expression of potential fusion proteins Analysis of expression of potential fusion proteins by the notifier is restricted to a bioinformatics analysis of the junction regions of the characterised MON810 insert. Recent findings indicate that a number of RNAs of fusion proteins between cry1Ab insert sequences and 3´flanking sequences originating from a HECT E3 ligase gene are indeed transcribed in MON810 (Rosati et al., 2008). The report is mentioned on the internet site of the notifier (http://www.monsanto.de/biotechnologie/biotechnews.php). We request that additional data on the nature of identified fusion RNAs is submitted and the relevance of findings in Rosati et al. (2008) is discussed.	The Rosati paper (2008) reported that translation of an RNA from the truncated *cry1Ab*-antisense HECT fusion gene results in a protein that contains 2 extra amino acids in addition to the Cry1Ab protein. This is also mentioned in the renewal application and reported in MSL0020709. The Rosati paper reported a possible fusion protein (18 additional amino acids to the *cry1Ab* component). The mRNA giving rise to this putative fusion protein probably originates from alternative splicing. However, bioinformatics showed that translation of the *cryAb1* RNAs does not give rise to fusion proteins with significant homology to known protein domains. It was not possible to amplify sequences from the insertion locus in the non GM maize control using primers from the 5'and 3'flanking sequence of the MON810 maize insert. The MON810 insert has likely resulted in DNA rearrangements or insertion of additional DNA. However, bioinformatics reveals no matches of putative fusion proteins encoded by the junction between the e35S promoter and the 5' flanking sequence with known allergens or toxins.
Austria	Ministry for Health Family and Youth	D, 04 Information on how the GM plant differs from the recipient plant in: …	No new data on agronomic parameters on MON810 were presented by the notifier. The notifier simply refers to agronomic observations during field trials and to commercial experience since 1997 but fails to submit any specific data on the agronomic behaviour of this GMO. Such failure to present any data is unacceptable as it contradicts the risk assessment principle of Directive 2001/18/EC. Annex II of Directive 2001/18/EC clearly requires that the "results of	See section 3.2.3*: The EFSA GMO Panel has already assessed the agronomic and phenotypic characteristics of maize MON810 in relation to an appropriate non-GM maize control having a comparable genetic background in connection with giving its opinions on several stacked events (EFSA, 2005a,b,c,d,e). The information available in the present renewal application gives no reason to change the opinion that maize MON810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with exception of the insect*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			adequate research into the potential risk in the deliberate release or placing on the market should be taken into account along with any clearly documented comparable experience". As experience has been gathered with MON810 cultivation in several EU member states, this experience should be clearly documented by the notifier with respect to the agronomic behaviour of this GMO.	*resistance conferred by the Cry1Ab protein.* The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of the plant.
Austria	Ministry for Health Family and Youth	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	For the demonstration of phenotypic stability similar data compared to the original notification were submitted. For the assessment of genetic stability of the modification Southern Blot data for 2 samples from MON810 plants derived from F1 and F4 generations are included. However the data consisting of Southern Blot experiments with single probes are insufficient for a comprehensive assessment and their representation in the technical dossier is of inferior quality (see Fig. 18 technical dossier p. 61). Therefore we request that additional significant data for a demonstration of genetic stability are submitted by the notifier with regard to the number of individual plants investigated and the varieties of MON810 which are assessed. It is requested that the lines of MON810 developed for application in Europe are assessed by methods which are superior for assessing changes to the modification, like multiple detection methods, i.e. as developed by Singh et al (2007), in addition to significant Southern Blot data. Such data should be submitted in a quality, which is adequate for an assessment by the competent authorities.	The integrity of the insert originally described in 1995 and 2001 was confirmed by the recent study of 2007, indicating stability of the insert.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry for Health Family and Youth	D, 07.03 Selection of compounds for analysis	(Sanders 1994; Sanders 1995) The field trials in the USA as well as in Europe were conducted during one year only, although weather conditions can significantly influence contents and Bt expression patterns (Hang Nguyen Thu (2004). Changes in expression is relevant since low Bt expression levels could result in faster resistance development of the target pests. No detailed information on the agricultural performance or weather conditions were presented. The contents are presented as means and ranges, therefore no detailed comparisons can be made. We request further analysis of data from individual sites. In the USA field trials 8 mean contents of amino acids were significantly higher in the GM corn, indicating differences in the N metabolism. Both test corn lines as utilised in the European trials showed less protein and a higher carbohydrate content as compared to the data from the USA. In the European trials only 2 different amino acids showed significant differences (lower values for the GM corn). The compositional data of the MON810 progeny lines are only presented as ranges, which makes it impossible to analyse them in any detail. Generally protein contents were higher for the locations in France, whereas dry matter content was considerably higher for the locations in Italy (Techn. Dos. [3] Table 13). Although all values are within the historical range, these differences reflect the metabolic reaction of the plant which can be important with regard to agricultural performance or resistance to pests and pathogens and should be investigated further. The Cry1AB expression levels also differed: in the USA samples were higher in the leaves and lower in the grain as compared to the European samples. The data submitted by the notifier were however different to data from the	The GMO Panel considered total compositional data supplied by the applicant which have become available since the original authorization (see, Section 4.1.2) and concludes that maize MON810 is compositionally equivalent to the non-GM counterparts MON 820 and MON 818 and to other conventional maize varieties except for the presence of the Cry1Ab protein. The Panel is not aware of any new compositional data that will lead to reassessment of its previous opinions.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			measurements published by Nguyen (2004), with lower values for both plant parts. These few examples show that site and year influence the compositional results. Therefore field trials should be performed over more than one growing seasons on differing sites to gain a deeper insight into composition patterns under different growing conditions (e.g. draught) and reduce the level of uncertainty with regard to changes in contents and expression levels.	
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	History of safe use and consumption "Since 1997, more than 65 million hectares have been cultivated and no toxic, allergenic or other harmful effects to human health or the environment have been reported" (Techn. Dossier [3], 7.8.1). However the notifier did not submit scientific data on long-term feeding tests with farm animals including breeding behaviour and assessment of more than a single generation of animals fed transgenic material. Under these circumstances anecdotal evidence based on farmer experiences hint at potential adverse effects (Riddle, 2002). E. g. in 2002 US farmers have reported breeding problems in farrows, when fed 100% with Bt corn. It is requested that these reports are further investigated. The notifier further argues that the "… history of safety is further supported by nearly 45 years of safe use of microbial Bt formulations (containing similar Cry1Ab) on a variety of crops." (Techn. Dossier [3], page 91). It is however not appropriate to compare pesticidal Bt toxin formulations with the agricultural use of Bt plants. The formulations contain a mixture of protoxins produced by the Bacillus thuringiensis which are applied to the plants at short times and are known to degrade within hours of their use since they are highly sensitive to light.	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization show that MON810 maize and its products are as safe to the experimental animals as traditional maize and its products.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Whereas in MON810 the transgenic Bt toxin is expressed as a synthetic and truncated version of the natural gene and is expressed in all parts of the plant during the whole vegetation period.	
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	Whole feed toxicity studies 13 week feeding Study in Rats with Grain from YieldGard (MON 810) Corn Grain (DK 551 Bt) Preceded by a 1 week baseline food consumption determination with PMI certified rodent diet #5002. (Lemen and Dudek 2001) There is a lack of information about the expression level of the transgene in the corn which could be done by quantitative PCR. Further quantification of the Cry1Ab is of special interest as there are fluctuations in protein expression over the harvests. The study design was set up for one test corn at two levels, a near isogenic control corn at two levels and six reference diets at one level. This study design with historical and reference ranges is very uncommon in feeding studies as slight differences might be masked. It is not clear in what form the diet was offered (pelleted, powdered?) but this is a crucial point as proteins and also Crystal proteins are heat labile. Two anaesthesia schemes were used for blood collection. This is not akzeptable as halothane has a different effect on blood parameters than CO2 and a comparison of blood parameters at week 6-7 and week 14 is not possible. At the same time the anatomical site of blood drawing differed (first retro-orbital sinus and later posterior vena cava). This is a major shortcoming since haematology parameters will differ by the anatomical site itself. Thus test data from week 6-7 and week 14 are again not comparable. The Bonferroni inequality might be too strong for the data correction as the test product related differences are rather slight. As	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization show that MON810 maize and its products are as safe to the experimental animals as traditional maize and its products.

Application EFSA-GMO-RX-MON810 (20.1a cultivation)				ANNEX G
Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			one lot was contaminated with DK551 Bt a new lot was used for the study. It is not clear if the data of nutritional equivalence, pesticide residues, mycotoxins etc, refer to the first or the second new lot. Chlordane was 5fold higher than the allowed specification of PMI diets but this was not considered as important for the authors. Fluctuations were reported in male and female body weight from the 33% test group to the control group. The authors don't see any coherence with the test article. Though it has to be mentioned that at group assignment no statistically significant difference was seen and, authors should consider the fact that different genotypes might differ on a stimulus. Thus fluctuations in body weights may indicate an effect of the test article. Moreover feed consumption differed which could be a question of palatability and as a consequence fluctuations in body weight might be explained. There are a number of statistically significant differences in haematology and chemistry data. These have to be regarded as test feed related since so many differences are very unlikely to occur by chance. More focus should be laid on test and control groups, neglecting the range of reference groups or historical ranges. The authors should furthermore discuss the significant differences identified in the submitted assessment. Further sentinel studies might bring more certainty about the safety of the product. Reproductive and developmental toxicity testing and repeated-dose toxicity testing should support the assessment of whole feed toxicity of MON810.	
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	Literature review (Aumaitre, A. 2004) The paper by Aumaitre (2004) is a review of studies published up to 2003, including feeding experiments with farm animals (pigs, poultry,	The Panel has analyzed the literature data relevant for the safety of MON810 maize for humans and animals that have become available since the original

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			cattle, lambs). The measured parameters were feed intake, weight gain, milk yield and nutritional equivalence expressed as feed conversion and/or digestibility of nutrients. Animal health and survival rate as well as milk and meat composition were considered. All these parameters showed no differences between the GMP and its non-GM counterpart. Furthermore no rDNA has ever been detected in any animal tissue or milk. Obviously Bt maize such as MON 810 is not acutely toxic to mammals; therefore these results are not surprising. But it is also clear, that any potential chronic effects cannot be found by short-term feeding of adult animals. The need to regain and keep up homeostasis would counteract any slight disturbances caused by a less than optimal diet. To ensure that no chronic health impacts are caused by GM feed components long-term feeding studies including several generations are necessary. The assumption, that GMPs are substantially equivalent "Since they have not been selected for a modification of their chemical composition" postulates, that drastic changes along the DNA such as unintended mutations and rearrangements have no influence on the plant's genetic expression pattern and denies potential pleiotropic effects, which can be elicited by weather conditions or be a site-related function. Even the expression pattern and intensity of the transgene differs yearly and regionally as mentioned above. Therefore in line with the precautionary principle no categorical, sustainable quality assurance can be given with certainty. Mazza et al. published feeding tests with pigs fed a MON 810 test diet in 2005 and is therefore not part of this review. But these authors did find small, albeit non-functional, tDNA (Cry1Ab fragments) in blood, liver, spleen	authorization. Relevant literature has been mentioned in the MON 810 opinion. Confirmation of no adverse effects induced by MON 810 maize has been obtained in similar 90-day feeding studies in rats fed maize with the MON810 event stacked by conventional breeding with EFSA 2005,a,2005b,2005c. Also see the review " Safety and nutritional assessment of GM plant and derived foods and feed,the role of animal feedindg trials." Food and Chemical Toxicology 46, S2-70.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			and kidney of the GM fed pigs (Mazza, R., Soave, M., Morlacchini, M., Piva, G., Marocco, A. (2005)). When interpreting feeding experiments storage time, grain drying and processing such as ensiling and pelleting of the GM test feed have to be taken into account, since the toxin content decreases over time and Cry1Ab might be sensitive to heat. Therefore the long list of feeding experiments with farm animals showing no GM effects in the literature study by Aumaitre (2004) has to be screened for the aforementioned feed characteristics to provide safe evidence.	
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	42 days Broiler feeding study (Taylor et al. 2001) Additionally to the test corn lines MON810, GA21xMON810 and the parental maize line 5 commercial hybrid maize lines were included to provide a range for comparisons. The main focus however should be on the direct comparison between the test line and the isogenic control line. The report furthermore includes no information on field test locations for production of the test material and growing parameters. All diets were pelleted with live steam addition involving heat treatment which could result in the denaturation of the test protein. This possibility is not discussed by the notifier. The test chicken had a higher feed intake and weighed more, but there was no difference in the feed efficiency. Only the adjusted feed efficiency was slightly better for the MON810-fed females. The contents of amino acids were elevated compared to the parental line (Table 1, page 45).	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization and reviewed by the Panel in the MON810 opinion show that MON810 maize and its products are as safe to the experimental animals as traditional maize and its products. The Panel does not consider long time animal feeding studies to be necessary. Feeding studies with several target animal species (broiler chickens, lactating diary cows, Atlantic salmon) have shown that Maize 810 is nutritionally equivalent to conventional non-GM maize.
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	Low exposure Oral toxicity studies are targeted on acute toxic effects, but give only limited information on the ability of a substance to cause harmful effects over an extended period. The route of administration does not resemble the	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization and reviewed by the Panel in the MON810 opinion show that MON810 maize and its

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			route of human or animal exposure. To determine potential chronic toxicity long time studies have to be performed. Whole feed conversion studies The study design of whole feed conversion studies does not include toxicological endpoints and therefore provides only an adaptability screening of the test animals to novel feeds.	products are as safe to the experimental animals as traditional maize and its products. The Panel does not consider long time animal feeding studies to be necessary. Feeding studies with several target animal species (broiler chickens, lactating diary cows, Atlantic salmon) have shown that Maize 810 is nutritionally equivalent to conventional non-GM maize.
Austria	Ministry for Health Family and Youth	D, 07.08 Toxicology	Oral toxicity studies (Naylor 1992) The cry1Ab gene encoding the full length Cry1Ab protein was introduced into E. coli and then reduced to the trypsin-resistant core. But the maize cells contain the codon-modified version of the transgene. While it is true that no differences concerning food consumption and cumulative body weight 7 days after the administration of the test substances were found, it is surprising that the test mice on average either lost weight or in any case did not gain weight. In detail the daily feed consumption ranged from under 5 g up to 16,8 g in general and in the highest dose group 50% of the female test mice consumed less than 5 g. Furthermore in most cases the amount of food consumed could not be corroborated by data concerning the weight development of test animals (e.g. female mouse 009 of the 400 mg/kg target dose group consumed 16,8 g/day, but lost 0,4 g weight; male mouse 010 of the highest dose group consumed 7,9 g/day and lost 1,2 g). The male mice generally consumed less than the female mice and the range within the female groups was much wider than in the male groups (e.g. in the 4000 mg/kg target dose group females ranged from 4.5 – 12 g/day, males from 4,2 – 7,9 g/day). With a view to these inconsistencies it is conrequested that larger test groups are examined to provide adequate data to further	The single dose acute toxicity study in mice is not designed for studying weight gain. However, as it lasted for 15 days data could be used in the assessment and it showed that the slight weight changes and food consumption during this period can be regarded as normal. The main conclusion from this study is still valid. There were no statistically significant differences in body weights, food consumption and mortality between the mice treated with Cry1Ab compared to the controls. No signs of systemic toxicity were observed.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			address these issues.	
Austria	Ministry for Health Family and Youth	D, 07.09 Allergenicity	Allergenicity Annex 3.5.a Cry1Ab McCoy, R. L. and A. Silvanovich (2004). "Bioinformatics analysis of the Cry1Ab protein produced in corn event MON810 utilizing the AD5, TOXIN5, and ALLPEPTIDES databases." Monsanto Technical Report MSL 19497. Annex 3.5.b Cry1Ab McClain, J. S. and A. Silvanovich (2007)."Updated bioinformatics evaluation of the Cry1Ab protein in corn MON 810 utilizing the AD7 allergen database." Monsanto Technical Report MSL 0020694. Bioinformatic analyses were performed to assess the potential for toxicity, allergenicity, or pharmacological activity of the Cry1Ab protein sequence produced in MON 810. No investigation of structure of allergens was performed though structure plays a major role in allergenicity. Safety assessment could be more profound if serum banks for testing of allergenicity would be used additionally.	See section 5.1.5 and 5.1.5.1 of the scientific opinion '*The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a*).'
Austria	Ministry for Health Family and Youth	D, 10.01 Persistence and invasiveness	Persistence and invasiveness, selective advantage/disadvantage The notifier refers to a phenotypic characterisation of maize MON810, which however has not been presented (see also point "agronomic evaluation"). The notifier further states that maize in general is not inherently persistent or invasive and that this is not different for maize MON810. However, this assumption should be supported by data since the notifier also refers to field trials (p 115 of technical dossier) in which it has been established that maize MON810 has not been altered phenotypically or in any agronomic, reproductive, survival or dissemination characteristics. These data should be submitted by the notifier. Equivalence between the	The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of the plant. See section 5.1 (toxicology) of the scientific opinion

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			microbial protein and the plant produced protein Equivalence of the introduced, plant produced protein and the microbial protein is crucial if test proteins derived from microbial sources are used assessing the sensitivity of target and non-target organisms to the protein in laboratory bioassays.. The equivalence of the microbial produced Cry1Ab toxin used in toxicological studies with target and nontarget organisms to the protein produced in maize MON810 has not been shown. The study submitted by the notifier characterising the microbial Cry1Ab protein (Berberich & Lee 1994) used protein extracts from a Bt cotton line (Line C81) as a comparator for the assessment of amino acid content, the terminal amino acid sequence and the Western Blot (reference to Perlak et al. 1990 using insect resistant cotton). Equivalency tests including a bioactivity assay should be done specifically with the relevant protein derived from MON810. Equivalence of the microbial and plant derived (MON810) Cry1Ab toxin by SDS-PAGE should be demonstrated. The SDS-PAGE of the microbial protein showed a major band at 63 kD and additional bands at approx. 20, 30 and 35 kD which were not observed in the HD-1t Standard (Berberich & Lee 1994).	
Austria	Ministry for Health Family and Youth	D, 10.04 Interactions between the GM plant and target organisms	Target organisms Some arguments by the notifier on the mechanism of action and specificity of the Cry1Ab toxin are outdated in view of current scientific knowledge. This information should be updated, especially as regards the mechanism of action of Cry toxins responsible for its toxic effect (Bravo et al. 2007, Jimenez-Juarez et al. 2007, Zhang et al. 2006) but also in respect to factors influencing the Cry-toxicity and insect susceptibility such as host plant composition (Ludlum et al. 1991), the	Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group in relation to interaction of the GM plant with NTOs. See section 6.1.3 (interaction of the GM plant with TOs) of the scientific opinion *"In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			presence of insect gut bacteria (Broderick et al. 2006) and insect age and condition (Huang et al. 1999, Hellmich et al. 2001, Reardon et al. 2004). Furthermore toxin interactions were not considered by the notifier. Synergistic interactions of Cry-toxins with other proteins have been described (Schnepf et al. 1998, Kashdan et al. 2001, Herman et al. 2002) and there are also indications for antagonistic interactions of Cry toxins with other toxic plant compounds (Santos et al. 1997). No data have been provided by the notifier to show the efficacy of the maize MON810 in particular for the target organisms, i.e. European corn borer and pink borers under different European environmental conditions. Due to differences in expression patterns under different environmental conditions (Nguyen & Jehle 2007) and regional or local differences in the sensitivity of pest species (Farinos et al. 2004), the efficacy of this maize is expected to differ. It is known that different lepidopteran species exhibit considerably different sensitivities towards the Cry toxins (MacIntosh et al. 1990), even differences between populations of a particular lepidopteran pest species vary significantly (Kranthi et al. 2004, Farinos et al. 2004). Thus data on baseline susceptibilities not only for target organisms of the MON810 (different corn borers) but also for possible secondary pests with regard to those agricultural areas where GM maize is expected to be planted in EU have to be submitted. Additionally other pest species which may be additional target organisms of maize MON810 should be evaluated and their sensitivities towards the Cry1Ab toxin reported, especially if they are also lepidopteran species.	*resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel recommends these species are considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires"*.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Austria	Ministry for Health Family and Youth	D, 10.05 Interactions of the GM plant with non-target organisms	Non-target organisms The notifier presented laboratory studies using a few surrogate taxa (honey bee, daphnia, earthworm, ladybird beetle, parasitic hymenoptera) which were fed the microbial isolated protein in acute toxicity tests using mortality as the only endpoint assessed. These lab studies give only little information on the safety of maize MON810. The ecological relevance of such studies is limited due to the type of test substance used (microbial versus plant-produced), the experimental test designs (no positive controls), and the toxicological endpoints used (acute response parameters only). Furthermore it has to be noted that such laboratory tests with non-target organisms have general limitations as additional environmental stresses are neglected (Marvier 2002, Andow & Hilbeck 2004, Lövei & Arpaia 2005). Thus laboratory ecotoxicological testing must be designed in a way to reflect the conditions in the field as much as possible with respect to pattern, duration and extent (dosage) of exposure. One of these studies using Daphnia magna (Graves & Swigert 1997) was carried out with pollen of Bt11 maize which is not the relevant GMO of this notification. Studies with isolated gene products are only one step in a tiered risk assessment approach as no pleiotropic effects are taken into account. The notifier has, however, not presented any results from field studies conducted during the extensive testing and commercial experience in the maize hybrids MON810 in different environments in the EU which, according to the notifier, did not reveal adverse effects on the environment. Simply stating that Cry1Ab expressing crops have no adverse effects on biodiversity, natural enemies and non-target arthropods and referring to a range of published studies without analysing the	ENV WG Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group in relation to interaction of the GM plant with NTOs. See section 6.1.4 (interaction of the GM plant with NTOs) of the scientific opinion

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			results of these studies in depth is not sufficient. A thorough environmental risk assessment approach must be composed of an exposure and effects assessment for individual taxa and ecological processes and must take several exposure pathways into account. Especially in view of the experience gained with the commercial cultivation MON810 in Spain, France and other EU countries, relevant data must be submitted.	
Austria	Ministry for Health Family and Youth	D, 10.05 Interactions of the GM plant with non-target organisms	With respect to nontarget organisms the notifier should present and discuss the following data for maize MON810: - Exposure assessment of relevant non-target organisms in representative maize fields in different EU maize growing regions (see Harwood et al. 2005, 2007, Zwahlen & Andow 2005) and effects assessment of those non-target species exposed to the Cry1Ab toxin under field conditions. - Assessment of laboratory studies using MON810 maize with particular focus on sublethal effects (Lövei & Arpaia 2005). - Assessment of field studies using MON810 maize with respect to the abundance of several relevant non-target taxa. Non-pesticide treated maize used as control should be included (Marvier et al. 2007). - Exposure assessment of representative non-target Lepidoptera including micro-Lepidoptera in the EU as well as species protected under Directive 92/43/EEC and regionally important species such as Nymphalidae, Lycaenidae, Hesperiidae and Pieridae (see e.g. Traxler et al. 2005, Heissenberger et al. 2003), including an assessment of non-cumulative and cumulative effects of Bt maize pollen and anthers over prolonged periods considering also sublethal effects (see e.g. Anderson et al. 2004, 2005, Vojtech et al. 2005, Dutton et al. 2005, Felke &	ENV WG Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group in relation to potential effects on NTOs. See section 6.1.4 (interaction of the GM plant with NTOs) of the scientific opinion See also section 6.1.6 (potential interaction with the abiotic environment and potential effects on biogeochemical processes) of the scientific opinion. The GMO Panel is of the opinion that "*potential effects on soil microorganisms and microbial communities due to maize MON810 if they occur, will be transient, minor and localised in different settings and are likely to be within the range currently cause by other agronomic and environmental factors*". Exposure assessment for some non target Lepidoptera species has been conducted by the GMO Panel based on a simulation model (see paragraph 6.1.4.2. b)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Langenbruch 2005). - Assessment of the potential persistence of the Cry1Ab toxin under representative European soil conditions including prolonged persistence (over at least two growing seasons). - Assessment of risks to soil organisms in laboratory, greenhouse and field tests (see Birch et al. 2007). - Assessment of nontarget pests, in particular Lepidoptera (e.g. Agrotis spp., Spodoptera sp.) in representative EU maize growing regions and an assessment of their sensitivity to the Cry1Ab toxin and their likelihood of resistance development to the Cry1Ab toxin. - Assessment of presence of the Cry1Ab toxin in relevant water bodies near maize growing areas and exposure of nontarget water organisms to the Cry1Ab protein (Rosi-Marshall et al. 2007, Bohn et al. 2008).	See section 6.1.6.1 (Persistence of Bt-proteins in soil) of the scientific opinion. See section 6.1.6.2 (microbiological effects in soil) of the scientific opinion. See section 6.1.4.4 (effects on non-target water-dwelling organisms) of the scientific opinion. "The EFSA GMO Panel is of the opinion that it is unlikely that the Cry1Ab protein in maize MON810 products would cause adverse effects on non-target water-dwelling organisms in the context of its proposed use".
Austria	Ministry for Health Family and Youth	D, 10.09 Impacts of the specific cultivation, management and harvesting…	Impact of the specific cultivation, management and harvesting techniques The notifier states that no specific cultivation, management or harvesting techniques are required for maize MON810 compared to conventional maize. Considerable experience with commercial cultivation of maize MON810 has been collected in Europe with respect to cultivation, management or harvesting techniques of this maize. Therefore the notifier is requested to provide relevant data gathered during cultivation of maize MON810 under different European agricultural conditions and considering alternative practices of controlling the target pests including pesticide and non-pesticide use. Positive agronomic aspects of cultivation of GM maize MON810, specifically the reduced use of chemical insecticides mentioned by the notifier are based on experiences made in the USA and cannot be assumed per se for EU maize production where insecticides are not applied in	See section 6.1.7 of the scientific opinion The GMO Panel is of the opinion that "*no new specific cultivation practices, management or harvesting techniques are associated to the cultivation of maize MON810. The only difference between maize MON810 and its conventional counterpart is due to fewer insecticide treatments needed to control lepidopteran target pests such as O. nubilalis and S. nonagroides (Gómez-Barbero et al., 2008a). As discussed above, the implementation of insect resistance management strategies is desirable to delay or prevent the potential evolution of insect resistance to Cry1Ab in lepidopteran target pest populations*".

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			similar ways and amounts to control the target species. Thus relevant data from Europe have to be provided by the notifier to support his conclusions.	
Austria	Ministry for Health Family and Youth	D, 12.02 Case-specific GM plant monitoring	Case-specific Monitoring The notifier proposes no case-specific post market monitoring actions due to conclusions of the ERA, except for an IRM plan. As discussed above the environmental risk assessment is not considered sufficient both with respect to the lack of data in general as well as regarding the lack of data in particular derived from different European conditions, thus clearly contradicting the case-to-case and region-by-region principle of Directive 2001/18/EC. The notifier should make available relevant data based on the commercial planting of maize MON810 in selected EU member states. Therefore no conclusions can be drawn for the potential negative effects of this GMO on European environments. The notifier presents monitoring reports for insect resistance from 2003 and 2004 in Spain (Annex 2a), in Czech Republic, France, Germany, Portugal and Spain in 2005 (Annex 2b) and in Czech Republic, France, Germany, Portugal, Slovakia and Spain in 2006 (Annex 3b). For the IRM plan a refuge size of 20% is considered appropriate by the notifier based on the assumption that a more fragmented landscape and diverse cropping conditions in the EU makes the current refuge requirements in the USA highly generous for EU conditions. This conclusion is based on theoretical assumptions rather than scientific facts. Additionally, a rough comparison of EU countries and the US with respect to farm numbers and percentage of land dedicated to agriculture does not take into consideration that maize cropland is usually clustered in certain	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC*". *"The EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel also recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires".* See section 6.1.3 of the scientific opinion The GMO Panel indicates that the reasons for implementing the *refugia* on farms where the Bt-maize area is greater than 5ha are: the high fragmentation of the European agricultural landscape; the lack of

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			areas within countries. Furthermore it is unclear why only farmers planting more than 5 ha of Bt maize MON810 are obliged to plant a refuge area. No scientific reasoning is provided for this requirement. This threshold is argued with economic terms rather than based on scientific arguments and with the argumentation that only a small proportion of the total maize area in the EU is cultivated on small farms with less than 5 ha. At the same time it is stated that the "less than 5 ha farms" represent a significant proportion of the maize farmers in the EU thus contradicting the previous statement. The assumption that farms planting less than 5 ha Bt maize will be bordered by other crops, barriers or fallow land might not be correct in areas with intensive and clustered maize cultivation and small farm sizes. Such maize production areas are of high relevance in certain EU Member States (Pascher & Dolezel 2005). In Austria farms with less than 5 ha account for approximately 20 % of Austrian farms (BMLFUW 2005). Implementation of the refuge The notifer states that the implementation of the IRM plan was assessed in a farmers survey conducted in Spain by ANTAMA and by the general surveillance in 2006 in six countries. It is clear that only a very coarse geographic resolution was achieved with this method as only 8,5% of the Bt maize MON810 area of the countries selected was monitored. The farmers selected to answer the questionnaire were thus not representative for the Bt maize growing area in some countries (e.g. Spain, France, Table 5 and Figure 6 in Annex 3). It is not indicated if and how compliance of farmers was assessed in regions where no questionnaires were used. Additionally, the results show a low compliance to refuge requirements for farmers who are	economic feasibility for providing *refugia* on farms with less than 5ha Bt-maize; and the negligible risk of resistance development in Bt-maize areas smaller than 5ha.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			required to grow a refuge in Spain. The notifier should propose a strategy to improve compliance to the IRM plan. The notifier states that in parallel with resistance monitoring complaints by farmers about the lack of efficacy of MON810 were also addressed (based on results of the ANTAMA survey in Spain). However, it is likely that such a survey based on qualitative parameters would indicate only major changes in the efficacy of Bt maize MON810. The notifier should indicate what change of efficacy (loss of efficacy) can be assessed by this method and indicate the likelihood of detection of such a change by the method proposed.	
Austria	Ministry for Health Family and Youth	D, 12.03 General Surveillance of the impact of the GM plant	With respect to the farm questionnaires the following additional information has to be provided by the notifier: - What criteria will be used for the selection of the subset of farmers which will use the questionnaires and how will be ensured that a representative sample of MON810 growers will be selected (including the statistical methodology for farmer selection)? - How will the questionnaires be validated, i.e. be ensured that the variables assessed actually measure a change (e.g. change in weed infestation)? - What baselines will be used and how they will be established in particular in view of different eco-agronomic characteristics and agricultural practices throughout maize growing regions in the EU. - What effect size will be monitored by the proposed questionnaire? For the parameters "plant diseases" and "weed control" the proposed questionnaire does not differentiate between different diseases or weed species. Also soil fertility depends highly on the amount of fertiliser use and cannot be estimated reliably by the "historical knowledge" of the farmer but rather needs scientific analyses of specific	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			scientific parameters. - How will be ensured that people involved will have the relevant education/knowledge to be able to identify and describe unanticipated adverse effects of MON810 maize? In the Annex attached the notifier refers to a manual for assisting farmers with filling in the questionnaires. This manual should be attached to the monitoring report. In summary, the monitoring plan for MON810 provided by the notifier lacks scientific strength with respect to the methods proposed. The use of farmer questionnaires as the sole method for general surveillance is not considered suitable for the assessment of unexpected environmental effects of maize MON810.	*by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Austria	Ministry for Health Family and Youth	D, 12.03 General Surveillance of the impact of the GM plant	General Surveillance Monitoring for unexpected adverse effects of the GMO on the farm level by using farm questionnaires is the sole monitoring responsibility proposed by the notifier. The notifier aims at shifting responsibility for monitoring activities and reporting to the national level and to external monitoring networks. This is not in agreement with the legal obligations as the responsibility of General Surveillance activities is with the notifier (Annex VII to Directive 2001/18/EC, Lit. 1.6). Individual member states may carry out additional monitoring activities if they consider it appropriate. Furthermore, to involve routine surveillance networks on an ad hoc basis only in case adverse effects are reported is not appropriate. Existing networks must be involved before GMO cultivation and it must be ensured that relevant data are collected by these networks and are available to the notifier. The notifier proposes the use of farmer questionnaires which will be provided to a selected range of farmers with experience in the	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed*

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			cultivation of MON810 maize. While questionnaires may be useful for compliance assessments they must be considered an inadequate strategy for the general surveillance of environmental effects which need thorough scientific assessment strategies rather than "general interview formats". The proposed questionnaires presented by the notifier do not cover environmental effects of the cultivation of Bt maize MON810 as required by Annex VII of Directive 2001/18/EC. The notifier is thus required to update the General Surveillance plan with the following information: - Information on how potential environmental effects will be covered in the GS plan. - Information if and how existing networks or established monitoring systems collecting ecological or environmental parameters in different member states will be integrated into the GS plan. - Information on the evaluation if the data collected by these existing networks are suitable to detect potential adverse effects of GM maize MON810 cultivation. - Information on the agreement of external networks to provide relevant data to the notifier. - Information on existing networks for monitoring effects on human/animal health to be used in the GS plan. It has to be questioned whether certain parameters can be assessed on the basis of individual knowledge of farmers rather than by scientific measurements. It is unclear how parameters which are not recorded during the cultivation period or at harvest (such as yield) can be assessed by the farmer, especially if detection is difficult as such (e.g. due to clustered distribution of pest, weeds) or if the identification of organisms needs specialised scientific knowledge (e.g. pest species identification). As farmers usually do not have expert taxonomic knowledge it has to be	*by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			explained by the notifier how the discrimination of diseases and pest species will be achieved. Some of these assessments will also need the use of laboratory equipment for determination. An assessment of parameters on a qualitative scale only, as done in the questionnaires, will result in different answers than "as usual" only if strong deviations from the "standard" situation occur (e.g. pest outbreaks). Deviations from the "standard" situation may also occur due to specific local conditions. In order to get a picture of the regional conditions and potential effects at a regional scale, an evaluation at a regional level of the assessed parameters must be considered.	
Belgium	Belgian Biosafety Advisory Council	A. General information	Point 7: the existence of a General Surveillance (GS) monitoring plan and Insect resistance management (IRM) plan should be mentioned.	(comment from a Member State)
Belgium	Belgian Biosafety Advisory Council	B. Information relating to (a) the recipient or (b) parental plants	Section B.1. : Information relating to the name of recipient is correct and complete. Nevertheless Tthe breeding origin of the MON810 should be specified (inbred pure line or population…). Section B.2., B3, B4: Scientific information about maize pollen dissemination and probable cross-pollination improved since 1998. As an application for renewal, the report should have mentioned new European scientific studies: Messean et al. (2006), Brunet (2006), Klein et al. (2003), Sanvido et al. (2007), Mazzoncini et al. (2007), … There is still a need for pollen dispersal monitoring. Survivability of maize in Europe is very limited but should be monitored in Southern Europe where winter mean temperatures are close to 15°C (South West Spain and SW Sicily). Brunet Y. 2006. Dispersion du pollen de maïs à longue distance : sources, transport, dépôt. Premier séminaire de restitution du programme ANR-OGM. Organismes	See section 6.1.2.2 of the scientific opinion The GMO Panel "*does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the fitness of hybrids and backcross progeny as well as exposure to non target organisms*". The GMO Panel concludes that "*the likelihood of unintended environmental effects due to the establishment and survival of maize MON810 will be no different to that of traditionally bred maize*".

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			génétiquement modifiés : aspects socio-économiques, alimentaires et environnementaux, 14 & 15 décembre, 2006, Paris, France, 61-64. Klein E.K, Lavigne C., Foueillassar X., Gouyon P.-H., Laredo C. 2003. Corn pollen dispersal : Quasi-mechanistic models and field experiments. Ecological Monographs. 73:131-150. Mazzoncini M., Balducci E., Gorelli S., Russ R., Brunori G. 2007. Coexistence scenarios between GM and GM-free corn in Tuscany region (Italy). Third International Conference on Coexistence between Genetically Modified (GM) and non-GM based agricultural supply chains, Seville, Spain 20&21 November, 2007. 295-296. Messean A., Bloc D., Richard-Molard M., Verdier J-L;, Gasquez J., Colbach N. 2006b. Impact du développement des plantes transgéniques dans les systèmes de culture. Premier séminaire de restitution du programme ANR-OGM. Organismes génétiquement modifiés : aspects socio-économiques, alimentaires et environnementaux, 14 & 15 décembre, 2006, Paris, France, 75-80. Sanvido O., Widmer F., Winzeler M, Bigler F. 2007. Scientific criteria for the evaluation of cross-fertilisation to define isolation distances for transgenic maize cultivation. Third International Conference on Coexistence between Genetically Modified (GM) and non-GM based agricultural supply chains; Seville, Spain 20&21 November, 2007. 97-100.	
Belgium	Belgian Biosafety Advisory Council	D, 03 Information on the expression of the insert	Pollen is a major plant part to be in contact with non-target insects. Why Monsanto didn't Monsanto complete the report with accurate pollen Cry protein levels? Other measurements are missing: toxin concentration in roots, tiller, ear leaf, silk and cob.	The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				the plant. See section 6.1.4.3.a (effects on non-target Lepidoptera, hazard characterisation) of the scientific opinion.
Belgium	Belgian Biosafety Advisory Council	D, 07.01 Comparative assessment	Production of material for comparative assessment. These presented data have already been reviewed before, by the EFSA GMO panel. Some important remarks and observations : 1. The range of nutrients covered is limited in comparison to similar dossiers. Information on mineral composition is rather limited and restricted to calcium and phosphorous. Vitamins are completely absent in the analysis. The importance of particular minerals and vitamins is substantial in food and feed. If maize is used as animal feed, minor nutrients like minerals and vitamins are generally added to concentrates; hence low concentrations in the maize may be overcome by these supplements. In case maize is used as a human food, minerals and vitamins play an important role, especially for particular consumer groups, among others consumers with a high intake of maize derived foods. 2. Maize is known to be rather sensitive to particular moulds, with the risk of production of mycotoxins. The dossier does not deal with this, although quite a lot of scientific information is available.	Analyses carried out on materials from maize MON810, including stacked events where maize MON810 was one of the parental lines, and their comparators indicate that maize MON810 is compositionally, phenotypically and agronomically equivalent to the non-GM maize counterparts and conventional maize, except for the introduced transgenic trait.
Belgium	Belgian Biosafety Advisory Council	D, 07.01 Comparative assessment	The results presented have been issued in 1994-1995. Does it mean that neither assessment nor monitoring has been carried out since that time?	See above. In addition, the toxicological and nutritional data on maize MON810 and appropriate non-GM control maize published during the last ten years confirm that these maize varieties have comparable influence on the test systems. Therefore, the EFSA GMO Panel is of the opinion that maize MON810 is as safe as its non-GM comparators and that the overall allergenicity of the whole plant is not changed through the genetic modification.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Belgium	Belgian Biosafety Advisory Council	D, 07.03 Selection of compounds for analysis	The CrY1Ab protein was produced by recombinant E. coli (P. 92-96 of Technical dossier), because it may be practically impossible to obtain a sufficient amount of plant derived protein. It has been mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins (Freese and 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.	The risk assessment approach undertaken in this issue is described in detail in the Guidance Document of the GMO Panel (2006) and its update (2008).
Belgium	Belgian Biosafety Advisory Council	D, 07.04 Agronomic traits	The information on the variability in the results is not straightforward available in the study with broilers (Taylor, 2001).	The GMO Panel concluded in its risk assessment by taking into account all the available information.
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 2 Chowdhury et al. (2003) reported that only traces of Cry1Ab survived the passage through the gastrointestinal tract of calves. This was confirmed by the fact that Lutz et al. (2005) found that Cry1Ab protein was degraded during digestion in cows. However, small fragments of Cry1Ab were detected in blood, liver, spleen and kidney of animals MON810 maize (Mazza et al., 2005). What is the medium or long term effect of this ? Chowdhury, E H, Shimada, N, Murata, H, Mikami, O, Sultana, P, Miyazaki, S, Nakajima, Y, Yoshioka, M, Hirai, N, Yamanaka, N 2003. Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. Veterinary and Human Toxicology 45: 72-74. Lutz, B., Wiedemann, S., Einspanier, R., Mayer, J., Albrecht, C. 2005. Degradation of Cry1Ab Protein from Genetically Modified Maize in the Bovine Gastrointestinal Tract. J. Agric. Food Chem., 53, 1453 -1456.	See the assessment of the GMO Panel on this issue in section 4.2.3.1(c) and (d)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Mazza, R., Soave, M., Morlacchini, M., Piva, G., Marocco, A. 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Research 14: 775-784.	
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 3 The study on Ladybird beetles (Hoxter, 1992b) does not provide information on the variability within the results so that the power of the analysis cannot be evaluated, which is also the case for the study on earthworms (Palmer, 1995). The number of animals per treatment is sufficient to find differences in the experiments with rats (Lemen and Dudek, 2001) and with mice (Naylor, 1992), but it is not the case for the study referred to as Monsanto Company (1996).	The GMO Panel concluded in its risk assessment by taking into account all the available information.
Belgium	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 1 At this moment, there seems to be no direct toxicological, nor ecotoxicological danger. Nevertheless, keeping in mind the precautionnary principle, no massive cultivation can be allowed as long as major doubts remain. Besides permanent monitoring of existing fields, major efforts are needed to generate valuable scientific data. Taking the additional information - concerning possible environmental effects - into consideration, some remarks are important • "The Bt maize produces 1500-2000 times as much Bt-toxin as is released through a single treatment in conventional crop protection, with the chemical called DIPEL, which contains Bt toxin." (1) "Other experiments have found that the residues of Bt plants are slower to decompose than their isogenic lines. Some 8% of the toxin produced by the plant remained in the field after harvesting. Indeed, a substantial share of this active toxin quantity could be identified in the soil 11 months later." (EFSA, 2005). . . The dossier should be completed with available information re the environmental impact of these	Se section 6.1.6.1 of the scientific opinion "*The potential accumulation of plant-produced Cry1Ab proteins in soil following repeated and large-scale cultivation of Bt-maize has been studied….. Despite the fact that Cry proteins can bind rapidly on clay minerals and humic substances, there is no evidence for accumulation of the Cry1Ab protein in soils in the field, even after 3 years of continuous cultivation of Bt-crops (e.g., Baumgarte and Tebbe, 2005; Marchetti et al., 2007; Hönemann et al., 2008)".* Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group in relation to potential effects on NTOs. See section 6.1.4 (interaction of the GM plant with NTOs) of the scientific opinion "*The EFSA GMO Panel has no reason to consider that maize MON810 will cause reductions to pollinating insects that are significantly greater from those caused*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			facts. • "Impact of Bt toxin on non-target organisms" (EFSA,2004) Permanent monitoring of existing fields and new scientific research should be conducted, in order to have first-class data on which appropriate decisions can be based. Further comments in D.9.4. • "Impact of MON810 maize on the large-scale beekeeping industry in Greece" (EFSA,2006) : The question that should be answered is whether bees are sensitive to this kind of toxin. Little literature is available and does not provide a clear answer to the problem. Immediate action should be undertaken to clear this item. The EFSA Journal (2004) 78, 1-13 The EFSA Journal (2005) 228, 1-14 The EFSA Journal (2006) 411, 1-26	*by conventional farming*".
Belgium	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	The applicant states that Cry1Ab is only a small part of the total protein content as an argument to confirm Cry1Ab as being non allergenic. However, only the titration of the protein of interest is valuable, not the determination of its relative content. In this respect, the levels of Cry1Ab are described at around 0.3 µg/g in the maize grain. This means that the ingestion of 300 g of non-concentrated maize-derived food product gives 90 µg of Cry1Ab, which lies in the lowest levels currently observed to be able to elicit allergic reactions. Simulated gastric fluid and simulated intestinal fluid (P.107-109, Technical dossier) were used to test allergenicity. However, Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Assessment of allergenicity of the whole GM plant. This has not been evaluated in the application. The reviewer wishes to emphasize that the rationale of this section is to evaluate, due to the introduction of the new traits, possible changes in the	See section 5.1.4 of the scientific opinion '*The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation, or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a).*'

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			allergenicity of the recipient plant when this plant is known as an allergenic source. Although not frequent, food allergy to maize exists and allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002, Weichel et al. 2006). The introduction and expression in the plant of Cry1Ab might interfere with other maize proteins, including allergens, and modify their expression levels. Care must be taken that food allergy to maize grain does not become more frequent due to the introduction of new traits and the interferences thereof. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified MON810 maize grains. Patient IgE binding to maize grain extract or titration of known major allergens of maize should be carried out for the GMO and natural counterpart. Given that the application also deals with cultivation in the E.U, another concern is maize pollen allergy. Although literature on that subject is scarce, allergy to maize pollen is well known in the allergy outpatient departments of the clinics and of the independent allergologists. It results from cross-reactivity with grass pollen, and is a major allergy problem in children living near maize fields. The most known cross-reacting allergens are Zea m 1 and Zea m 13, that cross-react with the group 1 and 13 allergens of grasses (Petersen et al. 2006). Therefore, the expression level of those major allergens should be determined in the pollen of genetically modified maize MON810. Bannon,G., Fu, T.J., Kimber, I., Hinton, D.M. 2003. Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124. Herman, R.A., Storer, N.P., Gao, Y. 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			114: 1154-1157. Pasini et al. Allergy 2002; 57:98-106 Pastorello et al. J Allergy Clin Immunol 2003; 112:775-83 Petersen et al. Proteomics 2006;6:6317-25 Weichel et al. Allergy 2006;61:128-35	
Belgium	Belgian Biosafety Advisory Council	D, 07.10 Nutritional assessment of GM food/feed	P.65, Table 10 (Technical dossier) tryptophan concentration in MON 810 is different from MON 818, although means are identical, but ranges are somewhat different. It looks like 0.6 is not the mean for MON818 with a range from 0.4 to 0.6; This may be verified. Compositional data for protein, fat, ash, ADF, NDF, fat, carbohydrates and dry matter for MON 810 were similar to the control, MON 820. How is dry matter in Table (Technical dossier)14 expressed? Units are lacking.	See section 5.1.5. Nutritional data on maize MON810 and appropriate non-GM maize control published during the last ten years confirm that these maize varieties have comparable influence on the test systems.
Belgium	Belgian Biosafety Advisory Council	D, 10.04 Interactions between the GM plant and target organisms	Baseline susceptibility and resistance allele frequency of the ECB is not uniform and depends on the sampling structure (population and region) (Meise and Langenbruch, 2007). Variations in toxin expression according to the plant age and environmental conditions can disturb the "high dose" strategy (Dutton et al., 2004). In addition, the monitoring of Bt expression shows that concentrations vary strongly between different plant individuals of Mon810 (Nguyen et al., 2007).So more studies should still be done to reduce uncertainty about resistance acquisition. As the risk of a resistance outbreak related to ECB and Sesamia is probable, IRM plans must be carefully respected in the scope of the Industry IRM Working Group (Alcalde et al., 2007). Toxin resistance is a major threat in the environmental risk assessment and recent results show that it is appearing in another species in Africa. As published recently (Van Rensburg, 2007), toxin resistance may evolve and present a tangible risk which must be	The EFSA GMO Panel considers that appropriate insect resistance management strategies are capable of delaying possible onset of resistance in field conditions. However, the EFSA GMO Panel advises that the potential evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			addressed with a specific monitoring. In that study, which has been conducted after reports of severe damage caused by Busseola fusca (stem borer), substantial numbers of larvae from the Bt derived population survived over the entire period of the bioassay. Alcalde E., Amijee F., Blanche G., Bremer C., Fernandez S., Garcia-alonso M., Holt K., Legris G., Novillo C., Schlotter P., Storer N., Tinland B. 2007. Insect resistance monitoring for Bt Maize cultivation in the EU : Proposal from the industry IRM working group. J. Verbr. Lebensm. 2, supplement 1:47-49. Dutton A., Klein H., Romeis J, Bigler F. 2002. Uptake of Bt-toxin by herbivores on transgenic maize and consequences for the predator Chrysoperla carnea. Ecol. Entomol. 27:441-447. Meise T., Langenbruch G.A. 2007. Susceptibility of German populations of the Corn Borer Ostrinia nubilalis (Lepidoptera :Pyralidae) to a Bacillus thuringiensis endotoxin. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 59 (12):297-301. Van Rensburg J.B.J. 2007. First report of field resistance by the stem borer, Busseola fusca (Fuller) to Bt-transgenic maize. South African Journal of Plant and Soil 24 (3):147-151.	
Belgium	Belgian Biosafety Advisory Council	D, 10.05 Interactions of the GM plant with non-target organisms	References for D.10.05 (follows) Harwood, J.D., Wallin, W.G. & Obrycki, J.J. (2005). Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Mol. Ecol. 14: 2815-2823. Harwood, J.D., Samson, R.A. & Obrycki, J.J. (2006). No evidence for the uptake of Cry1Ab Bt-endotoxins by the generalist predator Scarites subterraneus (Coleoptera: Carabidae) in laboratory and field experiments. Biocontrol Sci. Technol. 16: 377-388. Hilbeck, A., Baumgartner, M., Fried, P.M. & Bigler, F. (1998). Effects of transgenic Bacillus thuringiensis corn-	-

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			fed prey on mortality and development time of immature Chrysoperla carnea (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480-487. Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A. & Bigler, F. (1999). Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator Chrysoperla carnea. Entomol. Exp. Appl. 91: 305-316. Lövei, G.L. & Arpaia, S. (2005). The impact of transgenic plants on natural enemies: a critical review of laboratory studies. Entomol. Exp. Appl. 114: 1-14 Meissle, M., Vojtech, E. & Poppy, G.M. (2005). Effects of Bt maize-fed prey on the generalist predator Poecilus cupreus. Transgenic Res. 14: 123-132. Miller, H. I., Morandini, P. & Ammann, K. (2008). Is biotechnology a victim of anti-science bias in scientific journals? Trends in Biotechnol. 26: 122-125. Obrist, L.B., Dutton, A., Albajes, R. & Bigler, F. (2006). Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecol. Entomol. 31: 143-154. Parrott, W. (2008). Study of Bt impact on caddisflies overstates its conclusions: Response to Rosi-Marshall et al. PNAS DOI 10.1073/pnas.0711284105. Pilcher, C.D., Rice, M.E. & Obrycki, J.J. (2005). Impact of transgenic Bacillus thuringiensis corn and crop phenology on five nontarget arthropods. Environ. Entomol. 34: 1302-1316. Rodrigo-Simon, A., de Maagd, R.A., Avilla, C., Bakker, P.L., Molthoff, J., Gonzalez-Zamora, J.E. & Ferre, J. (2006). Lack of detrimental effects of Bacillus thuringiensis Cry toxins on the insect predator Chrysoperla carnea: a toxicological, histopathological, and biochemical analysis. Appl. Environ. Microbiol. 72: 1595-1603. Romeis, J., Dutton, A. & Bigler, F. (2004). Bacillus thuringiensis (Cry1Ab) toxin has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens)	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			(Neuroptera: Chrysopidae). J. Insect Physiol. 50: 175-183. Romeis, J., Meissle, M. & Bigler, F. (2006). Transgenic crops expressing Bacillus thuringiensis toxins and biological control. Nature Biotechnol. 24: 63-71. Rosi-Marshall, E.J., Tank, J.L., Royer, T.V., Whiles, M.R., Evans-White, M., Chambers, C., Griffiths, N.A., Pokelsek, J. & Stephen, M.L. (2007). Toxins in transgenic crop byproducts may affect headwater stream ecosystems. PNAS 104: 16204-16208. Vojtech, E., Meissle, M., & Poppy, G.M. (2005). Effects of Bt maize on the herbivore Spodoptera littoralis (Lepidoptera: Noctuidae) and the parasitoid Cotesia marginiventris (Hymenoptera: Braconidae). Transgenic Res. 14: 133-144. Zwahlen, C., Hilbeck, A., Howald, R. & Nentwig, W. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molec. Ecol. 12: 1077-1086.	
Belgium	Belgian Biosafety Advisory Council	D, 10.05 Interactions of the GM plant with non-target organisms	D.10.05 (references) Alvarez-Alfageme, F., Ferry, N., Castanera, P., Ortego, F. & Gatehouse A.M.R. (2008). Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator Stethorus punctillum Weise (Coleoptera: Coccinellidae). Transgenic Res. DOI 10.1007/s.11248-008-9177-4. Anderson, P.L., Hellmich, R.L., Prasifka, J.R. & Lewis, L.C. (2005). Effects on fitness and behavior of monarch butterfly larvae exposed to a combination of Cry1Ab-expressing corn anthers and pollen. Environ. Entomol. 34: 944-952. Beachy, R.N., Fedoroff, N.V., Goldberg, R.B., & McHughen A. (2008). The burden of proof: a response to Rosi-Marshall et al. PNAS DOI 10.1073/pnas.0711431105. Bøhn, T., Primicerio, R., Hessen, D.O. & Traavik, T. (2008). Reduced fitness of Daphnia magna fed a Bt-transgenic maize variety. Arch. Environ. Contam. Toxicol.	-

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			DOI 10.1007/s00244-008-9150-5 Bourguet, D., Chaufaux, J., Micoud, A., Delos, M., Naibo, B., Bombarde, F., Marque, G., Eychenne, N. & Pagliari, C. (2002). Ostrinia nubilalis parasitism and the field abundance of non-target insects in transgenic Bacillus thuringiensis corn (Zea mays). Environ. Biosafety Res. 1: 49-60. de la Poza, M., Pons, X., Farinos, G.P., Lopez, C., Ortego, F., Eizaguirre, M., Castanera, P. & Albajes, R. (2005). Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. Crop Protection 24: 677-684. Darvas, B., Kincses, J., Vajdics, Gy., Polgár, A. L., Juracsek, J., Ernst, A. & Székács, A. (2003). A DK-440-BTY (YIELDGARD) Bt-kukorica pollenjének hatása a nappali pávaszem, Inachis io lárvákra (Nyphalidae). [Effect of pollen of DK-440-BTY (YIELDGARD) Bt-maize on the larvae of Inachis io (Nymphalidae)] Abs. 49. Növényvédelmi Tudományos Napok (Eds. Kuroli G., Balázs K. és Szemessy Á.). p. 45. Darvas, B., Csóti, A., Gharib, A., Peregovits, L., Ronkay, L., Lauber, É. & Polgár A. L. (2004a). Adatok a Bt-kukoricapollen és védett lepkefajok larváinak magyarországi rizikóanalíziséhez. [Some data to the risk analysis of Bt-corn pollen and protected Lepidoptera species in Hungary.] Növényvédelem 40: 441-449. Darvas, B., Lauber, É., Polgár, L. A., Peregovits, L., Ronkay, L., Juracsek, J. & Székács, A. (2004b). Non-target effects of DK-440-BTY (YIELDGARD) Bt-corn. Abs. First Hungarian-Taiwanese Entomological Symposium, 11-12 October 2004, Budapest. p 5. Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M., Anderson, P.L. (2004). Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1ab-expressing corn during anthesis. Environ. Entomol. 33: 1116-1125.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Farinós, G.P., de la Poza, M., Hernández-Crespo, P., Ortego, F. & Castanera, P. (2008). Diversity and seasonal phenology of aboveground arthropods in conventional and transgenic maize crops in Central Spain. Biol. Control 44: 362-371. Ferry, N., Mulligan, E.A., Stewart, C.N., Tabashnik, B.E., Port, G.R. & Gatehouse, A.M. (2006). Prey-mediated effects of transgenic canola on a beneficial, non-target carabid beetle. Transgenic Res. 15: 501-514. Gathmann, A., Wirooks, L., Eckert, J., Schupman, I. (2006). Spatial distribution of Aglais urticae (L.) and its host plant Urtica dioica (L.) in an agricultural landscape: implication for Bt maize risk assessment and post-market monitoring. Environ. Biosafety Res. 5: 27-36.	
Belgium	Belgian Biosafety Advisory Council	D, 10.05 Interactions of the GM plant with non-target organisms	D.10.05 (follows) A study by Rosi-Marshall et al. (2007) published in the renowned journal PNAS showed that species of Trichoptera (caddisflies) occurring in headwater stream systems suffered negative effects of Cry1Ab from Bt maize in laboratory feeding trials. This study has been used to underline that an environmental risk assessment of Bt maize should also take into account water-dwelling insects. However, this paper has received serious criticism for its inappropriate methodology and unfounded conclusions in two letters to PNAS (Beachy et al., 2008; Parrott, 2008) and a scientific opinion paper in Trends in Biotechnology (Miller et al., 2008). The arguments laid out in these latter papers were entirely followed by EFSA (2007) and I can agree with these. Interestingly, however, negative effects of Cry1Ab were recently reported for another water dwelling organism, the water flea Daphnia magna (Bøhn et al., 2008); the laboratory experiments indicated a toxic effect rather than a lower	See paragraph 6.1.4.4. of the scientific opinion "*The EFSA GMO Panel is of the opinion that it is unlikely that the Cry1Ab protein in maize MON810 products would cause adverse effects on non-target water-dwelling organisms in the context of its proposed use*".

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			nutritional value of the Bt-maize. Furthermore: - Manachini et al. (2003) and Bourguet et al. (2002) found a decrease of the biocontrol function among specialist antagonist of target pest. - Daly et al. (2005) found a decrease of natural enemy abundance of Nabis sp. - Variable effects have been observed on earthworms (Zwahlen et al., 2003; Clark and Coats, 2006) (more details in D.9.8) The application for renewal states that (page 126) "Cry1Ab has a selective toxicity towards certain Lepidopteran pests but not against other orders". According to the arguments here-above, this statement should be moderated because there is an uncertainty about the Cry1Ab activity on some non-target organisms through the "gene x environment" interaction. So accurate monitoring plans must be put in place.	
Belgium	Belgian Biosafety Advisory Council	D, 10.05 Interactions of the GM plant with non-target organisms	D.10.05 (follows) The applicant refers to a number of field studies supporting the safety of Cry1Ab containing crops to a wide range of beneficial insects. The overall conclusion of these studies, that there are little differences in non target communities in Bt corn and non-Bt corn (where conventional insecticides are used), is well interpreted by the applicant. This conclusion is corroborated by two 3-year farm-scale studies in Spain focusing on arthropod predators (de la Poza et al., 2005; Farinos et al., 2008). On the other hand, a study by Pilcher et al. (2005) demonstrated lower densities of a specialist parasitoid in Bt corn plots, as a result of the lower abundance of its host, the target pest Ostrinia nubilalis. A study by Bourguet et al. (2002) also indicated that indirect effects on populations of certain more specific natural enemies of the different lepidopteran target pests would be expected if pest populations	Rearrangements of species assemblages at different trophic levels are commonly associated with any pest management practice. The EFSA GMO Panel is of the opinion that maize MON810 will not cause reductions to natural enemies that are significantly greater from those caused by conventional farming where pesticides are used to control maize borers. Exposure assessment for some non target Lepidoptera species has been conducted by the GMO Panel based on a simulation model (see paragraph 6.1.4.2.(b))

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			would be reduced as the result of the efficient control exerted by the expression of Cry-endotoxins (EFSA, 2006). Although similar effects are likely to occur also in non-transgenic maize where conventional pesticides are being used, I expect that the effect in transgenic maize may be less transient given that the toxin is expressed at very high levels (>99% level of efficacy, Pilcher et al. 2005) and continuously throughout the crop's cultivation period. Large-scale adoption of the transgenic maize may exacerbate these effects. Therefore, it is imperative to install sufficient refuges consisting of non-transgenic maize to avoid adverse impacts on these specific natural enemies. - Effects on non-pest lepidopterans feeding on maize would be expected, but the crop does not constitute an important resource of food for indigenous butterflies in Europe; pollen is only shed by Bt-maize plants during a limited window of time and it remains in the immediate vicinity of the crop, so possible adverse effects are expected to be transient and local. In the USA, mainly effects on the monarch butterfly (which does not occur in Europe) were studied. E.g. according to their results Prasifka et al. (2007) assume that "Monarch larvae exposed to Mon810 anthers behave differently and that ingestion may not be the only way Bt can affect non-target insects". Although some of these studies showed some adverse fitness effects, they were not considered likely to pose a significant risk to the monarch butterfly populations in North America (see Dively et al., 2004; Anderson et al., 2005 and references therein). Little is known on the distribution of European lepidopteran species in agricultural landscapes and their potential exposure to Bt maize (e.g., Gathmann et al., 2006). However, negative impacts on native	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			butterflies were reported by Darvas et al. (2003, 2004 a,b). According to latter studies Cry1Ab toxins may kill some 20% of hatching Inachis io caterpillars on nettle plants within 5 m of MON810 Bt maize. However, it should be stressed that: - these Hungarian papers and the data therein were inaccessible to me - two of these references are merely abstracts of symposia - none of these data were, as far as I could retrieve, ever subjected to peer review, or at least disseminated into the international scientific literature; this is all the more striking given the relevance of the reported findings.	
Belgium	Belgian Biosafety Advisory Council	D, 10.05 Interactions of the GM plant with non-target organisms	Many studies have demonstrated no or little direct effects of the Cry1Ab-endotoxin on non-target invertebrate organisms, including non-pest herbivores, pollinators and carnivorous natural enemies. Not surprisingly, Bt environment affects non-target fauna differently compared with non sprayed agro-system (Marvier et al., 2007). Only few studies have reported adverse effects of Cry1Ab toxin on non-target organisms. Lövei & Arpaia (2005) and Romeis et al. (2006) provide reviews of the literature on side effects of Cry proteins to beneficial arthropods. Laboratory studies by Hilbeck et al. (1998, 1999) revealed some negative effects on the predatory insect Chrysoperla carnea, but later studies showed that these adverse effects were not direct toxic effects but were mediated by (nutritional) quality of intoxicated prey, i.e. an indirect effect (Romeis et al., 2004; Rodrigo-Simon et al., 2006). Lower prey quality was also believed to be partly or wholly responsible for adverse effects observed in the generalist carabid predator Poecilus cupreus and the parasitoid Cotesia marginiventris offered prey or hosts that were	-

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			fed Bt maize (CryIAb) (Meissle et al., 2005; Vojtech et al., 2005); the experiments in Meissle et al. (2005) showed that the ground beetle did not avoid Bt-containing prey, which means that exposure in the field may occur. However, in a study on transgenic canola expressing CryIAc on another carabid beetle as a non-target indicator organism (Ferry et al., 2006), the results suggested that behavioural preferences of the predator (i.e., rejection of contaminated prey) would mitigate adverse indirect effects of reduced prey quality caused by consumption of Bt-canola plants. The degree of exposure suffered by non-target organisms has been the subject of different studies with variable outcome. The applicant may be criticized for only listing studies in the literature review in Annex 3.1 showing that exposure of non-target organisms is negligible or non-existent. For instance, the applicant refers to a study by Harwood et al. (2006) showing that there was no evidence for uptake of Cry1Ab endotoxins by a carabid predator in laboratory and field experiments. However, the applicant fails to mention other studies by the same authors that do indicate such uptake and suggest that the toxin may transfer into higher order trophic levels of food chains (Harwood et al., 2005, 2007). Similar findings (i.e., that Bt toxins can be transferred to predatory arthropods) were reported in a field study by Obrist et al. (2006). Likewise, Alvarez-Alfageme et al. (2008) showed that Cry1Ab toxin was transferred in undegraded form from Bt-maize to the predatory coccinellid Stethorus punctillum via its tetranychid prey, but that the predator did not suffer adverse effects from the toxin.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Belgium	Belgian Biosafety Advisory Council	D, 10.08 Effects on biogeochemical processes	Comment 2 The applicant dossier and the studies referred to therein have reported no or negligible effects of the expressed Cry1Ab proteins on detritivorous organisms living in and on the soil. For instance, there are no reported effects of the Cry1Ab toxin on the annelid Eisenia fetida (an epigaeic compost worm that is usually not found in maize fields) nor on the collembolan Folsomia candida. A number of studies have reported no negative effects of Cry1Ab expressing maize on other soil organisms (discussed in EFSA 2006). On the other hand, there are reports on sublethal effects of the Cry1Ab expressing GM maize on immatures and adults of the anecic earthworm Lumbricus terrestris when fed on litter of the GM maize, although the adverse effects could not be confirmed in higher-tier (small scale) field trials (Zwahlen et al., 2003). In a laboratory study, Vercesi et al. (2006) reported no detrimental effects of Bt maize residues on development and fecundity of the earthworm Aporrectodea caliginosa (which is a more relevant species in an agricultural setting), with the exception of a slight decrease in cocoon hatchability. However, the authors questioned whether this effect would have any ecological significance in the field. Wandeler et al. (2002) studied the consumption of Bt- and non-Bt-maize by the woodlouse Porcellio scaber and reported that the woodlouse fed less on the Bt maize than on the corresponding non-Bt control variety. They also found that the woodlouse was effectively exposed to the toxin, but made no mention of adverse effects on the organism. In a glasshouse experiment, Griffiths et al. (2006) reported some effects of Cry1Ab expressing maize on soil microbial and faunal communities but these effects were all minor and comparable to those of conventional ("current best practice")	-

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			insecticide treatments. It needs emphasis again that so far no published studies have described the consequences of long-term cultivation of Bt corn on earthworms and other soil dwelling animals. Griffiths, B.S., Caul, S., Thompson, J., Birch A.N.E., Scrimgeour, C., Cortet, J., Foggo, A., Hackett, C.A. & Krogh, P.H. (2006). Soil microbial and faunal community responses in Bt maize and insecticide in two soils. J. Environ. Qual. 35: 734-741. Vercesi, M.L., Krogh, P.H. & Holmstrup, M. (2005). Can Bacillus thuringiensis (Bt) corn residues and Bt-corn plants affect life-history traits in the earthworm Aporrectodea caliginosa? Appl. Soil Ecol. 32: 180-187. Wandeler, H., Bahylova, J. & Nentwig, W. (2002). Consumption of two Bt and six non-Bt corn varieties by the woodlouse Porcellio scaber. Basic Appl. Ecol. 3: 357-365. Zwahlen, C., Hilbeck, A., Howald, R. & Nentwig, W. (2003). Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molec. Ecol. 12: 1077-1086.	
Belgium	Belgian Biosafety Advisory Council	D, 10.08 Effects on biogeochemical processes	Comment 1 The role of cultivation practices on the impact of Bt in the environment hasn't been taken enough into account: - Fu et al.(2008) report the occurrence of interactions between inorganic salts (contained in mineral fertilisers) and Bt toxin adsorption that impact the fate of Bt toxins in the soil. - Icoz et stotzky (2008) report that microbial process play a major role in the dissipation of CryA toxins and this process depends, in turn, on soil type, seasons, cultivar, crop practices… Bt concentrations in the soil don't always decrease as faster as described in section D 9.5. Marchetti et al. (2007) report a DT50 of 10 or 11 days for sandy and clay soil, which is higher than the 1.5 day mentioned in the application for renewal. Additional	See section 6.1.6 of the scientific opinion The GMO Panel is of the opinion that "*no new specific cultivation practices, management or harvesting techniques are associated to the cultivation of maize MON810. The only difference between maize MON810 and its conventional counterpart is assigned to different pest management practices*".

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			concentrations of Bt toxins in the soil due to GM cultivation affects the GRAM+/GRAM- ratio compared with a non-GM crop (Xue et al., 2005). Most of the results published in this area are still preliminary (Evans et al. 2002; Zwahlen et al. 2003) and need more complements. Nevertheless, at this stage of the knowledge, current studies show no significant or acute detrimental effects of CryA toxins on soil microorganisms or soil microbiology. Evans HF.2002. Environmental impact of Bt exudates from roots of genetically modified plants. Defra Report EPG 1/5/156. Icoz I., Stotzky G. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology & Biochemistry 40 (3):599-586. Fu QL., Wang WQ., Hu HQ., Chen SW. 2008. Adsorption of the insecticidal protein of Bacillus thuringiensis subsp. Kurstaki by minerals: effects of inorganic salts. European Journal Of Sol Science 59 (2):216-221. Marchetti E., Accinelli C., Talamè V., Epifani R. 2007. Persistence of Cry toxins and cry genes from genetically modified plants in two agricultural soils. Agron. Sustain. Dev. 27:231-236. Xue K., Luo HF., Qi HY., Zhang HX. 2005. Changes in soil microbial community structure associated with two types of genetically engineered plants analysing by PLFA. Journal of Environmental Sciences 17 (1):130-134. Zwahlen C., Nentwig W., Bigler F., Hilbeck A. 2003. Effects of transgenic Bt corn litter on the earthworm Lumbricus terrestris. Molecular Ecology 12:1077-1086.	
Belgium	Belgian Biosafety Advisory Council	D, 10.09 Impacts of the specific cultivation, management and harvesting…	Mon810 is currently a reliable means to control ECB but, as mentioned in section D 10.04, there is a risk that target insects develop CryA toxin resistance in the medium or long term. The report states that Mon810 "reduces the use of	This is a comment from the MS.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			chemical insecticides and reduces applicator exposure to dangerous active ingredients" but doesn't mentions the number of insecticide sprays that can be saved. ECB and Sesamia are among the major pests in maize fields and may cause 13% yield losses. ECB reinforces its presence in southern France and progresses rapidly up north where two generations can grow in a season. In France, infestation hotspots are seen in the Rhone region and South-West. However, in most of the regions spray numbers are still low: in Poitou-Charentes in France (see Agreste Report, 2003), where an average larvae density is about 0,8 larvae/plant, only 42% of the maize fields are sprayed (mostly with pyrethroïds) and receive 1.4 sprays on average. Insecticide quantities, mostly Cypermethrin and Lamda Cyhalothrin, respectively average 0.08 and 0.01 kg/ha, which is not an exaggerated load for the environment in comparison with other crops. Similar statistics are found in Germany and Spain (Brookes, 2002). This information is confirmed by the Mon810 monitoring report (Schmidt and Tinland, 2006). In conclusion, the impact of the pesticide reduction due to Bt maize on human health or environment still remains weak and doesn't represent a major benefit. No reference is made about coexistence measures. However specific lay-out, spatial organisation and separate chain processing may be required in some areas (Messean, 2006b). These measures generate additional economic costs, which should be discussed. Mon810 should be recommended in a IPM management system, which should be detailed by the Monsanto Company. Agreste Report. 2003. Enquête pratiques culturales 2001. available on line : www : http.//draf.poitou-charentes.agriculture.gouv.fr Brookes G. (2002).	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			The farm level impact of using Bt maize in Spain http://www.bioportfolio.com/news/btmaizeinspainfinalreport16september.pdf Messean A., Bloc D., Richard-Molard M., Verdier J-L;, Gasquez J., Colbach N. 2006b. Impact du développement des plantes transgéniques dans les systèmes de culture. Premier séminaire de restitution du programme ANR-OGM. Organismes génétiquement modifiés : aspects socio-économiques, alimentaires et environnementaux, 14 & 15 décembre, 2006, Paris, France, 75-80. Schmidt K., Tinland B. 2006. Post market monitoring of Bt maize Mon810 in Europe-Survey with farm questionnaires in 2006. Monsanto.	
Belgium	Belgian Biosafety Advisory Council	D, 12.01 General	The applicants describe an insect resistance management (IRM) plan for case-specific monitoring of resistance development in corn rootworms that is generally based on methods that have up to now been widely accepted by the community of scientists and practitioners. However, care should be taken to meticulously implement and continuously evaluate and, if needed, adjust the recommended measures in the plan, particularly if large scale adoption of the Bt/herbicide tolerant maize would change existing cropping methods (e.g., related to crop rotation or tillage) possibly affecting abundances of the target pests, other herbivores and natural enemies. Also, some of the key assumptions for the high-dose refuge strategy which forms the basis of the environmental monitoring plan, may not be entirely fulfilled. Farinós et al. (2004) highlighted the need to adapt the insect resistance management strategies for certain areas. It has been shown that 1) Sesamia nonagrioides females mate before they move for oviposition, so that females emerging from	See section 6.1.3 of the scientific opinion "*If Bt-maize was adopted on a larger scale in a region, the risk of resistance development is likely to increase requiring specific refuge management measures. Since risk management is outside the remit of the EFSA GMO Panel, it is the responsibility of appropriate competent authorities in Member States to approve insect resistance management plans that are consistent with the environmental protection goals and biodiversity action plans in each Member State*"..

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			refuge would rarely mate with potential resistant moths emerging from Bt maize fields and vice versa, and 2) Ostrinia nubilalis mobility is also reduced before oviposition in irrigated maize fields, which corresponds to the agronomic practices of most maize growing areas in Spain. These findings need to be considered in the IRM plan. Dalecky et al. (2006) further pointed out that: - the behavioural ecology of stem borers, as related to the timing between dispersal and mating, may differ from species to species (and so may be different between Ostrinia nubilalis and Sesamia spp.) - the high dose refuge strategy, in which refuges are situated a few hundred meters from Bt maize fields, may not ensure complete mixing between susceptible and resistant Ostrinia nubilalis moths, because some pre-dispersal mating occurs for both males and females (up to about 57% of newly emerged females have been found to mate locally) - Bt-resistance alleles in resistant stem borers may be associated with fitness costs, decreasing dispersal and mating success; as such, intermixing between susceptible and resistant moths may be further compromised Eizaguirre et al. (2006) showed that in Sesamia nonagrioides: - a high proportion of larvae may move to plants in adjacent rows, favouring the survival of partially resistant individuals; this suggests that mixing of Bt and non-Bt maize in the same field would not be a recommendable strategy to delay resistance - surviving adults that have been exposed to sublethal concentrations of the Bt toxin have an asynchronous development compared to individuals originating from non Bt refuges which limits the random mating between susceptible and resistant individuals - individuals exposed to sub-lethal levels of Bt maize may have lower fitness (e.g., as related to their	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			responsiveness for diapause inducing and terminating cues) Although these studies show that the main prerequisite for random mating in the high-dose refuge strategy proposed in the resistance monitoring plan may not be fulfilled, there have been no field reports to date of resistance in lepidopteran stem borers towards Bt-maize expressing Cry1Ab, or other Cry toxins. Dalecky, A., Ponsard, S., Bailey, R. I., Pélissier, C. & Bourguet, D. (2006). Resistance evolution to Bt crops: predispersal mating of European corn borers. PLoS Biology 4: 1048-1057. Eizaguirre, M., Albajes, R., Lopez, C., Eras, J., Lumbierres B. & Pons, X. (2006). Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects. Transgenic Res. 15: 1-12. Farinós, G.P., de la Poza, M., Hernández-Crespo, P., Ortego, F. & Castanera, P. (2004) Resistance monitoring of field populations of the corn borers Sesamia nonagrioides and Ostrinia nubilalis after 5 years of Bt maize cultivation in Spain. Entomol. Exp. Appl. 110: 23-30	
Belgium	Belgian Biosafety Advisory Council	D, 12.02 Case-specific GM plant monitoring	1) Interactions between the GM plant and target organisms, case-specific monitoring with risk management. The IRM plan is complete. To delay the risk of insect resistance and reinforce the IR management, Mon810 should be introduced when a certain average pest pressure threshold (example: 0.8 larvae/plant) is reached in a given area and should not be adopted below (see Hochberg et al. (2006) for more details). 2) Additional case-specific monitoring without risk management Contradictory results (pointed in section 9.5 and 9.8) should be re-assessed by the scientific community (Itps, EFSA….) on a multi-disciplinary base, under standard process and methods. Hochberg M., Vacher C.,	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC"*. *"The EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel also*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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			Desquilbet M., Bourguet D., Ambec S., Lemarié S. 2006. Gestion de la résistance des insectes phytophages aux PGM. Premier séminaire de restitution du programme ANR-OGM. Organismes génétiquement modifiés : aspects socio-économiques, alimentaires et environnementaux, 14 & 15 décembre, 2006, Paris, France, 93-94.	*recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires".*
Belgium	Belgian Biosafety Advisory Council	D, 12.03 General Surveillance of the impact of the GM plant	Is there a parallel field/farm monitoring plan put in place by the public sector (INRA or others) or does field data rely only upon Monsanto monitoring network? This information doesn't appear clearly in the report. Farm questionnaire: not accurate enough for non-target arthropods ? - The observation data related to the monitoring character is quality based and leads sometimes to inaccurate results, whatever reliable the non parametric statistical analysis can be. - Data related to fertiliser application is not accurate and doesn't consider doses/ha. - Insecticides are simply mentioned without indication of doses, quantity or number of sprays during the agricultural season. This avoids the comparative evaluation of the biocide charge in the environment (a farmer can decrease the number of sprays but increase the quantity of insecticide). - Main weed species occurrence should be mentioned in comparison with non-Bt fields. - The questionnaire focuses mainly on target and non-target pests, when non-target neutral arthropods and beneficials are not. The observed data should be based on counts of bioindicators belonging to the different existing functional groups (ECB parasitoids, predators, neutral species, earthworms, amphibians, birds…) through sampling or trap monitoring, according to a precise protocol (see methods in Delos et al, 2006). Interactions between the GM plant and non-target organism Contradictory results have	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			been reported about predatory insects (see introduction in Marvier et al., 2007) because of deficient protocols or analysis. The interaction between Bt toxin and the quality of the target prey must be more deeply investigated and should be included in an ERA plan: would non-Bt starving preys induce the same effect as Bt infected preys? Uncertainties due to the difficulty of interpretations present in the conclusions of impact studies as seen in Candolfi et al. (2004), related to the impact of Bt toxin on lepidopteran, dipteran and hymenopteran species in the field in particular, should be cleared within an accurate monitoring plan. This plan could be assimilated to a case-specific monitoring, in the long term, to test the hypothesis of an indirect adverse effect of Bt toxin on the non-target fauna. Results of the 2006 monitoring report (Schmidt and Tinland, 2006) indicating a non-expected effect on Diptera and Araneae support this suggestion. Toxin expression Since the combination of non-biotic stresses may affect toxin concentration in the plant (Dutton et al., 2004), the results of the GS plan (target pest pressure, presence or absence of irrigation) should be systematically linked with local climatic data, to complete the monitoring of the efficacy of Mon810 on target insects and the high-dose & refuge IRM. Areas to be monitored (section 11.4.3.3.) There is an inconsistence at line 10-12: "the Glyphosate trait has utility in a wide range of agricultural environment. Therefore, the introduction of Mon810 is not confirmed to specific geographical zones" It should be: the Bt trait has utility…. Candolfi M.P., Brown K, Grimm C., Reber B., Schmidli H. 2004. A faunistic approach to assess potential side-effects of genetically modified Bt-corn on non-target arthropods under field conditions. Biocontrol Science and Technology 14:129-170.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Dutton A., D'Alessandro M., Romeis J., Bigler F. 2004. Assessing expression of Bt toxin (Cry1Ab) in transgenic maize under different environmental conditions. Bull. OILB SROP 27 (3):49-55 Marvier M., McCreedy C., Regetz J., Kareiva P. 2007. A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates. Science 316:1475-1477. Schmidt K., Tinland B. 2006. Post market monitoring of Bt maize Mon810 in Europe- Survey with farm questionnaires in 2006. Monsanto.	
Belgium	Belgian Biosafety Advisory Council	D, 12.06 Reporting the results of monitoring	The general surveillance is adapted for a broad agricultural survey focused on potential and additional benefit to the farming unit. On the other hand, it seems to be less adapted to environmental impacts. In order to address specific impact issues of Mon810, GM-fields should theoretically be compared with non-GM fields of the same farm or agrosystem. In the GS monitoring reports (2005 and 2006) presented in Annexe II of the dossier, data from small GM-fields (less than 5 ha, without obligation to plant refuge) have been processed with data from large GM-fields. Especially in the case of Spain, where refuges are not often respected and where Mon810 plantings are the majority (see the rate of all maize/Mon810 acreage), no direct comparison between Bt and non-Bt maize could be made in the same farming unit. Introduction of bias and misinterpretation of data may occur: - If the GM farmer made a comparison with one of its neighbours' non-GM field (without knowledge of the cropping history), - If the farmer refers to non-GM data from previous years. Some of the results of the GS reports should also be analysed and presented separately at a regional or national scale (highlighting treatment x local environment).	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			This is the case for the incidence of diseases, which are sometimes specific to a region. The interaction between Bt and the environment or agrosystem should be more often taken into account. Finally, only two general surveillance reports have been presented separately in the annexes (2005, 2006). It would have been interesting to assess to results of previous years and have them compiled in order to to study multi-annual trends.	See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
France	MEIE - DGCCRF	General comments	Malgré une présentation confuse des données dans le dossier technique, l'Agence Française de Sécurité Sanitaire des Aliments considère que : Ø l'analyse moléculaire du maïs portant l'événement MON810 caractérise l'événement de transformation, Ø l'analyse de composition ne met pas en évidence de différence significative compromettant l'équivalence en substance du maïs MON810 par rapport au maïs témoin et aux variétés de maïs conventionnelles, Ø l'étude de toxicité subchronique réalisée chez le rat pendant 90 jours ne met pas en évidence d'effets délétères liés à la consommation du maïs portant l'événement MON810, Ø l'étude d'alimentarité réalisée chez le poulet ne met pas en évidence de différences nutritionnelles entre le grain de maïs MON810 et le grain de maïs témoin. En conséquence, l'Agence Française de Sécurité Sanitaire des Aliments estime, qu'au regard des données présentées dans le dossier dont certaines ont été réactualisées et des nombreuses données publiées dans la littérature scientifique à comité de lecture (annexe 1), les maïs portant l'événement de transformation MON810 et leurs produits dérivés présentent le même niveau de sécurité sanitaire que les variétés de maïs conventionnelles et que leurs produits dérivés.	(this is a comment from a Member State) As can be seen in the conclusion of the scientific opinion, the GMO Panel considers that maize MON 810 is as nutritious and safe as conventional maize, more or less agreeing with the French Food Safety Agency.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			EN TRANSLATION Despite a muddled presentation of the data in the technical file, the French Food Safety Agency is of the following view: Ø the molecular analysis of maize carrying the MON810 event characterises the transformation event, Ø the composition analysis shows no significant difference compromising the substance equivalence of MON810 maize compared with the control maize and conventional maize varieties, Ø the subchronic toxicity study conducted on the rat for 90 days shows no deleterious effects associated with the consumption of maize carrying the MON810 event, Ø the feed safety study conducted on the chicken shows no nutritional differences between MON810 maize grain and the control maize grain. Consequently, the French Food Safety Agency believes that in the light of the data presented in the file, some of which has been updated, and the numerous data published in the peer-reviewed scientific literature (Annex 1), maize carrying the MON810 transformation event and their derived products offer the same level of safety to health as conventional maize varieties and their derived products.	
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	General comments	Suite à une évaluation sur le maïs MON810 conduite par le Comité de préfiguration d'une haute autorité sur les OGM, dont les conclusions ont été rendues le 9 janvier 2008, il apparaît que de nouveaux éléments scientifiques sont disponibles concernant l'impact de ce maïs sur l'environnement. Les commentaires transmis par l'autorité compétente sont des extraits de l'avis du comité de préfiguration. Cet avis n'est pas basé sur le dossier de renouvellement du maïs	(this is a comment from a Member State)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			MON810, qui n'était pas encore disponible, mais sur la littérature scientifique disponible à la date de l'avis. EN TRANSLATION Following an assessment of MON810 maize conducted by the Preliminary Committee of the High Authority on GMOs, whose conclusions were published on 9 January 2008, it emerges that new scientific information is available on the impact of this maize on the environment. The comments submitted by the competent authority are extracts from the opinion of the Preliminary Committee. This opinion is not based on the renewal application for maize MON810, which was not yet available, but on the scientific literature available at the date of the opinion.	
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	B, 04 Dissemination; (a) ways and extent (for example and estimation of how…	Le fait nouveau depuis 1998 concerne la caractérisation de la dispersion du pollen (Klein et coll, 2003 ; Rosi-Marshall et coll, 2007 ; Brunet 2006) (Kuest ; Chapela 2001) sur de grandes distances (kilométriques) (A. MESSEAN, 2006) liée notamment aux conditions et événements climatiques et aux milieux. Ces résultats ont conduit à démontrer l'impossibilité d'une absence de pollinisation croisée entre champs OGM et champs sans OGM à une échelle locale (petite région agricole) (A. MESSEAN, 2006). La dissémination de la toxine Bt et sa persistance ont été démontrées et dépendent de facteurs édaphiques, climatiques et du milieu (Icoz et Stostky ; 2007). EN TRANSLATION The new fact that has emerged since 1998 concerns the characterisation of pollen dispersion	See section 6.1.2.2 (plant to plant gene transfer) of the scientific opinion The GMO Panel "*does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the fitness of hybrids and backcross progeny as well as exposure to non target organisms*". The GMO Panel concludes that "*the likelihood of unintended environmental effects due to the establishment and survival of maize MON810 will be no different to that of traditionally bred maize*". See also the scientific opinion of the GMO Panel on the French safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm).

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			(Klein et coll, 2003; Rosi-Marshall et coll, 2007; Brunet 2006) (Kuest; Chapela 2001) over long distances (in kilometres) (A. MESSEAN, 2006), linked in particular to climate conditions and events and to environmental conditions. The results showed that the absence of cross-pollination between GMO fields and non-GMO fields was impossible on a local scale (small agricultural region) (A. MESSEAN, 2006). Dissemination of the Bt toxin and its persistence have been proven and depend on soil, climate and environmental factors (Icoz, Stostky; 2007	
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	D, 10.04 Interactions between the GM plant and target organisms	Sélection de souche résistante sur deux lépidoptères cibles secondaires (Huang et al, 2007 ; Van Rensburg, 2007). EN TRANSLATION Selection of resistant strain in two secondary target lepidoptera (Huang et al, 2007; Van Rensburg, 2007).	See section 6.1.4 (interaction of the GM plant with NTOs) of the scientific opinion. See also the scientific opinion of the GMO Panel on the French safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm).
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	D, 10.05 Interactions of the GM plant with non-target organisms	Des faits nouveaux confirment la possibilité d'effets toxiques avérés à long terme sur les lombrics (Zwalhen et al. 2003), les isopodes, les nématodes et sur les monarques (rhopalocères) (Hardwood et al. 2005, Prasifka et al. 2007 ; Dutton et al, 2005). L'exposition sur les populations naturelles de monarques reste très limitée (moins de 1%), notamment pour ces derniers via des effets comportementaux dommageables. (Marvier et al., 2007). Des publications démontrent la présence possible de la toxine Bt dans la chaine trophique (Obrist et al, 2006) ainsi qu'une persistance observée des molécules insecticides dans l'eau (Douville et al, 2006 ; Rosi-Marshall et al, 2007) ou dans les sédiments drainant d'une parcelle (plus de 20 à	See section 6.1.4 (interaction of the GM plant with NTOs) of the scientific opinion. See also the scientific opinion of the GMO Panel on the French safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm).

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Comments from National Competent Authorities under Directive 2001/18/EC				
			40 jours) (Ipoz, Stotsky, 2007), au contact des racines et dans le sol (Saxena et Stotzky, 2005 ; Mulder et al. 2006 ; Castaldini et al, 2005) avec une exposition des populations d'insectes (Griffith et al., 2006 ; Johnson et al, 2006) plus en amont des chaînes trophiques. Une analyse globale sur l'entomofaune non cible (Marvier et al 2007) démontre un effet des cultures de mais Bt sur quelques familles d'invertébrés. EN TRANSLATION New facts confirm the possibility of toxic effects emerging in the long term in earthworms (Zwalhen et al. 2003), isopodes, nematodes and monarch butterflies (Rhopalocere) (Hardwood et al. 2005, Prasifka et al. 2007; Dutton et al, 2005). Exposure of natural monarch butterfly populations is still very limited (under 1%), with the latter being exposed as a result of damaging behavioural effects (Marvier et al., 2007). Some publications indicate the potential presence of the Bt toxin in the trophic chain (Obrist et al, 2006) as well as the persistence of insecticide molecules in water (Douville et al, 2006; Rosi-Marshall et al, 2007) and in sediment draining from fields (over 20 to 40 days) (Ipoz, Stotsky, 2007), in contact with roots and in the soil (Saxena, Stotzky, 2005; Mulder et al. 2006; Castaldini et al, 2005,) with insect populations being exposed (Griffith et al., 2006; Johnson et al, 2006) upstream of the trophic chains. A global analysis of non-target entomofauna (Marvier et al 2007) shows that the Bt maize crops have an effect on some families of invertebrates.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	D, 10.08 Effects on biogeochemical processes	En outre, le comité souligne la nécessité d'approfondir l'évaluation des effets biologiques et microbiologiques de la dissémination ou de la persistance observée des molécules Bt ou du transgène dans le sol (plus de 200 jours) (Crecchio, Stotzky, 2001). EN TRANSLATION In addition, the Committee underlined the need for a thorough assessment of the biological and microbiological effects of dissemination and of the persistence of the Bt or transgene molecules in the soil (over 200 days) (Crecchio, Stotzky, 2001).	See section 6.1.6 (potential interaction with the abiotic and potential effects on biogeochemical processes) of the scientific opinion. See also the scientific opinion of the GMO Panel on the French safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm).
France	Ministère de l'Agriculture et de la Pêche/ Direction générale de l'alimentation	D, 12 Environmental Monitoring Plan	Le comité souligne l'importance d'un suivi en temps réel et sur du long terme des effets des cultures de plein champ du MON 810 sur la faune, la flore, la fonge, les écosystèmes, dans le cadre d'un programme de biovigilance. EN TRANSLATION The Committee emphasised the importance of real-time and long-term monitoring of the effects of MON810 outdoor crops on fauna, flora, fungi and ecosystems, in the context of a biomonitoring programme.	See section 6.2 (monitoring plan) of the scientific opinion
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	General comments	The German CA is of the opinion that the application for the renewal of the authorisation of MON 810 maize does not fully meet the requirements of the Regulation 1829/2003. It is desirable that the applicant adds updated information on the comparative assessment, the expression of Cry1Ab and the interaction of the GMO with non-target organisms and discusses these aspects in more detail. With regard to the inserted fragment the applicant should be	Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the potential effects on NTOs. Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM).

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			requested to clarify inconsistencies with information given in other context. The German CA is of the opinion that the applicant should consider to make use of already established monitoring networks in member states according to Annex VII, C. 3.2. of Directive 2001/18/EC for the implementation of the monitoring plan presented.	The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (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.
Germany	Federal Agency for Nature Conservation (BfN)	A. General information	Comments of the Federal Agency for Nature Conservation (BfN): The Federal Agency for Nature Conservation regards the data provided by the applicant as not sufficient to complete the environmental risk assessment. Although the application has been given the statement of validity substantial information has not been updated. Generally only limited information was provided to reflect the geographical or biogeographical regions of Europe where cultivation of MON810 maize seeks permission. The application is also incoherent since references to individual publications are mostly missing thus impeding the conclusions and its transparency by the applicant (e.g. 'specific information'). Major deficits of the application include the lack of a valid exposure analysis for non-target organisms and insufficient eco-toxcicity testing. With regard to new scientific findings tests should be amended to include more taxonomic groups of Lepidoptera and also focus stronger on aquatic organisms. Available data indicate a risk of MON810 cultivation for non-target Lepidoptera. This is particularly important for rare and endangered species. No data were submitted to allow a risk assessment with regard to the regional and local diversity of European habitats and protected areas which may be affected by the cultivation of MON810 maize. Therefore, a risk management should	Additional information has been requested to the applicant by the molecular characterisation group, food-feed group and environmental group. See section 6.1.4 (interaction of the GM plant with non-target organisms) of the scientific opinion. See also the scientific opinion of the GMO Panel on the French safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902156394.htm). During the evaluation of the risk assessment performed by the applicant, the GMO Panel requested the applicant to review and comment upon the studies with MON 810 published in the peer-reviewed literature since the authorisation in 1998.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			especially ensure the protection of endangered species and protected areas. Based on recent findings and implementing the precautionary principle MON810 maize should not be allowed to be cultivated close to inshore waters until the effects of Cry1Ab maize on caddisflies and Daphnia have been further investigated. If the present application should be approved, the Federal Agency for Nature Conservation recommends the following risk management measures to minimize risk for the environment: • MON810 maize should be cultivated only in regions where the target organism (Ostrinia nubilalis) is known to cause serious pest problems. It should be up to the Member States to define these regions in their respective territory. The objective is to minimize the exposure of the environment to Cry1Ab. This measure is also in accordance with the principles of Integrated Pest Management to use pesticides (here the incorporated insecticidal protein) only in combination with a threshold for pest infestation. • Cultivation of MON810 maize should not be allowed in and close to Natura 2000 sites. Exemptions can be made by the competent national authorities after an additional environmental impact assessment for the specific site according to Article 6 paragraph 3 Directive 92/43/EEC has been carried out • The authorization does not prejudice regulations under national law of the Member States providing for the protection of species and habitats, especially case-by-case requirements and administrative decisions according to provisions laid down in Directive 92/43/EEC and 79/409/EEC.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Germany	Federal Agency for Nature Conservation (BfN)	D, 02 Information on the sequences actually inserted or deleted	Comments of the Federal Agency for Nature Conservation (BfN): The study provided by Monsanto (Scalon et al., 2007) concerning molecular characterisation is in some parts in contradiction to newer publications. These publications document that the integration of the transgene into the maize genome caused complex recombinations, deletions and insertions in parts of the maize-genome (Rosati et al., 2008, Singh et al.; 2008). To clarify these inconsistencies further molecular characterisation of the Mon810-genome is indispensable. It is proposed to provide comparable data on at least three different approved European varieties. Rosati, A., Bogani, P. Santarlasci, A. & Buiatti, M. (2008) Characterisation of 3´transgene insertion site and derived mRNAs in Mon810 YieldGard maize, Plant Mol. Biol. Singh, C. K., Abhishek, O. Kamle, S. & Kachru, D. N. (2008): Assessment of cry1Ab transgene cassette in commercial Bt corn MON810: Gene, Event, Construct & GMO concurrent characterisation www.natureprotocols.com/2007/10/23/assessment_of_cry1ab_transgene.php	The Rosati paper (2008) reported that translation of an RNA from the truncated *cry1Ab*-antisense HECT fusion gene results in a protein that contains 2 extra amino acids in addition to the Cry1Ab protein. This is also mentioned in the renewal application and reported in MSL0020709. The Rosati paper reported a possible fusion protein (18 additional amino acids to the *cry1Ab* component). The mRNA giving rise to this putative fusion protein probably originates from alternative splicing. However, bioinformatics showed that translation of the *cryAb1* RNAs does not give rise to fusion proteins with significant homology to known protein domains. It was not possible to amplify sequences from the insertion locus in the non GM maize control using primers from the 5'and 3'flanking sequence of the MON810 maize insert. The MON810 insert has likely resulted in DNA rearrangements or insertion of additional DNA. However, bioinformatics reveals no matches of putative fusion proteins encoded by the junction between the e35S promoter and the 5' flanking sequence with known allergens or toxins.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 02 Information on the sequences actually inserted or deleted	The German CA is of the opinion that molecular characterisation provided with the application is sufficient and consistent with published literature. However, there are discrepancies regarding the construct used for transformation as described in the application and in the Monsanto held patent US 6713259. The applicant is requested to clarify which plasmid was actually used to transform MON810 and why the patent states a different plasmid as source of the transferred construct.	It has been clarified with the applicant that PV-ZMBK07 and pMON15772 are identical plasmids. Monsanto is currently evaluating the incorrect information in the patent application.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Germany	Federal Agency for Nature Conservation (BfN)	D, 03 Information on the expression of the insert	Comments of the Federal Agency for Nature Conservation (BfN): Expression data should permit reliable estimates on the quantity of expression in different plant tissues and its variability. Updated data on the composition of MON 810 including the analysis of all available data concerning expression of Cry 1Ab should be mandatory. Because expression may be influenced by abiotic factors (e.g. climate, soil), by agricultural practice (e.g. fertilization; Bruns & Abel 2003, 2007) and by the genetic background (variability in different varieties) data representative for the environments and the varieties used in the MS are requested. Bruns,H.A. & Abel,C.A. (2003) Nitrogen fertility effects on Bt delta-endotoxin and nitrogen concentrations of maize during-early growth. Agron J, 95, 207-211. Bruns,H.A. & Abel,C.A. (2007) Effects of nitrogen fertility on Bt endotoxin levels in maize. Journal of Entomological Science, 42, 35-43.	The original application provided sufficient data on protein expression levels. In 1994, field trials were conducted at six locations distributed throughout the major U.S. maize growing region representing a variety of environmental conditions. In 1995, five field trials were conducted within the major maize growing regions of France and Italy. With regard to Cry1Ab, the protein levels were similar for plants grown in the United States and European field trials over two consecutive generations. The levels of Cry1Ab detected did not raise any safety concerns and there is no new data to question this opinion.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 03 Information on the expression of the insert	Cry1Ab protein expression has been determined for different plant tissues obtained from 6 US field sites in 1994 and from 5 European field sites in 1995. However, no updated information on the levels of expression of Cry1Ab is presented in the application. Updated information is only provided indirectly by reference to applications of stacked events including MON810. The applicant provides no review or comparison of these data but cites the respective EFSA opinions on the respective individual applications. Considering the long time-span of commercial application of MON 810, the presented data sets are rather scarce. The applicant should be asked whether up-to-date expression analyses of progeny of MON 810 are available and whether data sets representative for MON 810 progeny in	The original data provided was comprehensive and did not indicate any safety concerns with regard to protein expression levels.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			commercial use could be provided. The applicant should be requested to provide a review on available expression data on event MON810 single and in stacked events.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.01 Comparative assessment	Comments of the Federal Agency for Nature Conservation (BfN): As with information on the expression of the insert updated data on the composition of MON 810 should be mandatory. To obtain reliable data we recommend sampling plant material during a minimum of three growing seasons and at six locations in the EU representing different environmental conditions. The environmental conditions should be documented and provided with the application. A summarizing statistical analysis should address the between-site variation of all parameters.	The extent of field trials in applications supplied from 2004 and onwards are described in the Guidance of GMO PANEL. At the time the original notification on Maise MON810 was suppliead within Europeean Commission, the extent of field trials required were less defined.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 07.01 Comparative assessment	To produce material for the comparative assessment of MON 810, the applicant performed field trials in the USA at 6 sites in the 1994 growing season and in France at 3 sites in the 1995 growing season. In these trials, MON 810 plots were grown as single replicates. In France, merely up to 25 seeds of MON 810 were planted per site. Further information on the design of the US trials is not given. Data are not presented by site, but as range over all sites in the USA or France, respectively. In the technical dossier, the applicant states that further field trials with progeny of MON 810 were performed in Italy and France in the 1995 growing season. Results are presented in tables 13-15. The corresponding study report is missing and should be provided by the applicant. Considering the long time-span of commercial application of MON 810, the presented data sets are rather scarce. The applicant should be asked whether up-to-date compositional analyses of progeny of MON 810 are available and whether data sets	See section 4 of the scientific opinion

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			representative for MON 810 progeny in commercial use could be provided.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.04 Agronomic traits	Comments of the Federal Agency for Nature Conservation (BfN): No information has been presented by the applicant on agronomic traits. The mere indication that "agronomic observations performed during field trials with MON810 supports a conclusion that from an agronomic and phenotypic (morphological) point of view, MON810 is equivalent to conventional maize, except for the introduced lepidopteran-protection trait" needs to be supported by field data. This analysis should be in accordance with good scientific practice.	MON810 agronomic traits have been assessed in stacked events (EFSA 2005abcde) and the Panel concludes that from agronomic point of view, MON810 agronomic characteristics do not deviate from those of currently grown non-GM maize. The applicant has provided to the Panel an updated list of references which have become available since the original authorization and the Panel has analyzed all relevant new data. Also see annual monitoring reports from the applicant (no adverse agronomic characters identified). The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the determination of the agronomic characteristics of the plant.
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Pollinators Only parts of the above functional groups were tested with regard to MON810 maize. Because of the important role of pollinators (economically and ecosystem-service) we explicitly point out that studies on pollinators focused exclusively on the honey bee. However, other organisms than honey bees use maize pollen and should be screened prior to the renewal of authorization of MON810 maize. To complete the e.r.a. the applicant is asked to submit further studies with bumble bees or solitary bees and adult hoverflies (Diptera:	Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the potential effects on NTOs. See section 6.1.4.3 (effects on pollinating insects) of the scientific opinion which also considers additional information available in the scientific literature.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Syrphidae). Field studies effects of MON810 maize on pollinators are virtually absent.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Eco-toxicological testing Due to the experimental designs employed and the low statistical power the information which can be drawn from the tests submitted for MON810 maize is very limited. This criticism has been ad-dressed in a more general form by the scientific community (see also Hilbeck et al. 2000; Andow & Hilbeck 2004; Lövei & Arpaia 2005). Because of the importance of eco-toxicity testing in the risk assessment process it is important to stress that GMO-tests should take the following points into account: • Test should include exposure via plant tissues, also in a multi-trophic context • Tests should verify the Bt-content in the plant material used • Uptake of the Bttoxin by the test organisms should be demonstrated • Test with microbial produced toxin should consider several dose groups • The statistical power of the test should be analysed. With the studies presented the detec-tion of significant differences was partly hampered by the experimental design (e.g. number of replicates or sample size) • The route of exposure should be realistic and ecologically meaningful • Test species should be selected in consideration of the European receiving environments. The selection criteria applied should be transparent and consider all European bio-geographic regions where the GMO maize will be grown. • All relevant functional groups in the receiving ecosystems should be tested The eco-toxicological information in application EFSA/GMO/RX/MON810 has not been updated since 1998. Studies submitted include the following test-organisms: Lacewing (WL-92-	Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the potential effects on NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion which also considers additional information available in the scientific literature.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			155), para-sitic hymenoptera (WL-92-157), honey bee larvae (IRC-91-ANA-12), honey bee adult (IRC-91-ANA-13), ladybird beetle (WL-92-156), springtail (XX-97-064), earthworm (WL-95-281), and daph-nia (WL-96-322). All but two of the studies were carried out with microbial produced toxins. While using microbial produced toxins has its merits (when the comparability of the toxicity is verified) to simulate exposure with the toxins higher than the concentration of Bt-toxins in plant tissue such experiments cannot substitute experiments with plant tissues. These are essential to consider un-expected effects due to the genetic modification or the expression of the toxins in the plant. The applicant is therefore asked to submit additional studies using plant material of MON810 maize. Test species should be selected to suit the different European ecosystems. A thorough test-ing of non-target organisms belonging to the same taxonomic order as the target organism is es-sential to estimate the lowest NOEC with regard to rare or endangered species which are pro-tected under EU and national law. To assess adverse non-target effects of MON810 maize the following functional groups should be considered: 1. Non-target organisms belonging to the same taxonomic group as the target organisms 2. Pollinators and pollen-feeding insects 3. Primary consumers including secondary pests 4. Predators 5. Parasitoids 6. Soil organisms 7. Water organisms The selection of test species should be representative and cover all European biogeographical regions where maize may be cultivated. Andow,D.A. & Hilbeck,A. (2004) Science-based risk assessment for non-target effects of transgenic crops. BioScience, 54, 637-649. Hilbeck,A., Meyer,M. & Raps,A. (2000) Review on Non-Target Organisms and Bt-Plants.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			EcoStrat EcoStrat GmbH / Greenpeace international. Lövei, G. L., Arpaia, S. (2005) The impact of transgenic plants on natural enemies: a critical review of labora-tory studies. Entomologia Experimentalis et Applicata 114, 1-14.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Literature Exposure Baumgarte,S. & Tebbe,C.C. (2005) Field studies on the environmental fate of the Cry1Ab Bt-toxin produced by transgenic maize (MON810) and its effect on bacterial communities in the maize rhizosphere. Mo-lecular Ecology, 14, 2539-2551. Crecchio, C. & Stotzky, G. (1998) Insecticidal activity and biodegradation of the toxin from Bacillus thur-ingiensis subsp. kurstaki bound to humic acids from soil. - Soil Biol. Biochem. 30: 463-470. Crecchio, C. & Stotzky, G. (2001) Biodegradation and insecticidal activity of the toxin from Bacillus thur-ingiensis subsp. kurstaki bound on complexes of montmorillonite-humic-acids-Al hydroxy-polymers. - Soil Biol. Biochem. 33: 573-581. Douville,M., Gagné,F., Blaise,C. & André,C. (2007) Occurrence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Sa-fety, 66, 195-203. Harwood,J.D., Wallin,W.G. & Obrycki,J.J. (2005) Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem. Molecular Ecol-ogy, 14, 2815-2823. Harwood,J.D., Samson,R.A. & Obrycki,J.J. (2007) Temporal detection of Cry1Ab-endotoxins in coccinellid predators from fields of Bacillus thuringiensis corn. Bulletin of Entomological Research, 97, 643-648. Hofmann, F. (2007) Kurzgutachten zur Abschätzung der Maispollendeposition in Relation zur Entfernung	(-)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			von Maispollenquellen mittels technischem Pollensammler PMF (Estimation of maize-pollen deposition with the pollen mass filter (PMF) in relation to the distance from pollen sources. (unpublished report; Fe-deral Agency for Nature Conservation, Germany). Hopkins,D.W. & Gregorich,E.G. (2004): Detection and decay of the Bt endotoxin in soil from a field trial with genetically modified maize. European Journal of Soil Science, 54, 793-800. Obrist,L.B., Klein,H., Dutton,A. & Bigler,F. (2005) Effects of Bt maize on Frankliniella tenuicornis and expo-sure of thrips predators to prey-mediated Bt toxin. Entomologia Experimentalis et Applicata, 115, 409-416. Obrist,L.B., Dutton,A., Albajes,R. & Bigler,F. (2006) Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields. Ecological Entomology, 31, 143-154. Ohlfest,J.R., Jesse,L.C.H., Jurenka,R. & Obrycki,J.J. (2002) Stability of insecticidal CryIAb protein in trans-genic B t corn pollen exposed to UV irradiation. J Kans Entomol Soc, 75, 48-51. Rosi-Marshall,E.J., Tank,L.J., Royer,T.V., Whiles,M.R., Evans-White,M., Chambers,C., Griffiths,N.A., Pokel-sek,J. & Stephen,M.L. (2007): Toxins in transgenic crop byproducts may affect headwater stream eco-systems. Proceedings of the National Academy of Science USA, 104, 16204-16208. Tapp, H. & Stotzky, G. (1998) Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. Soil Biol. Biochem. 30: 471-476. VDI-Richtlinie 4330 Blatt 3 (2007): Monitoring der Wirkungen von gentechnisch veränderten Organsimen (GVO) – Pollenmonitoring – Technische Pollensammlung mit Pollenmassenfilter PMF und Sigma-2–Sammler. VDI-Handbuch Biotechnologie, VDI/DIN-Handbuch Reinhaltung der Luft, Bd. 1a . Beuth-Verlag, Berlin Zwahlen,C. & Andow,D.A.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			(2005) Field evidence for the exposure of ground beetles to Cry1Ab from trans-genic corn. Environmental Biosafety Research, 4, 113-117. Zwahlen,C., Hilbeck,A., Gugerli,P. & Nentwig,W. (2003): Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology, 12, 765-775.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Exposure The applicant is asked to submit a detailed analysis of exposure of potential non-target organisms (NTO) to the Bt-protein expressed by MON810 maize. This analysis should cover all environmental media (including water and water sediment) both in areas where maize is cultivated and in adja-cent areas (including semi-natural and natural areas). The cry1Ab gene inserted in MON810 is controlled by a constitutive promotor leading to expres-sion of the Bt toxin in all plant tissues over the vegetation period. Because the Bt toxin is known to move into higher trophic levels (Harwood et al. 2005; Zwahlen & Andow 2005; Obrist et al. 2006; Harwood et al. 2007) these also have to be considered in the e.r.a. In this context Bt concentra-tions in higher trophic levels can be equal or higher than in the plant tissues (e.g. Dutton et al. 2002 for data on Tetranychus urticae; Obrist et al. 2006 for data on Frankiniella tenuicornis). Latest ob-servations by Obrist et al. (2006) showed that faeces of F. tenuicornis contain Cry1Ab concentra-tions several times higher than in maize leaves (Bt11). Obrist et al (2005) conclude that arthropods can be exposed to Bt toxin when licking dew or honeydew on plant tissues affected by thrips fae-ces. In summary available data indicate that long-term exposure to Cry1Ab will occur in the field for NTO belonging to different functional and taxonomic groups of the food web. Pollen	Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the potential effects on NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion which also considers additional information available in the scientific literature.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			deposition is an important aspect analyzing exposure because Cry1Ab toxin from MON810 maize will be carried in the surrounding landscape via pollen. While a range of studies examined pollen deposition close to field margins with several, non-standardized methods (e.g. sticky traps), recent data obtained with pollen traps especially designed for GMO pollen monitoring (2007; VDI guidelines 4330 Blatt 3) shed more light and took larger distances into account (up to several hun-dred meters) (Hofmann 2007). The analysis of Hofmann is based on field data from Germany and Switzerland during the years 2001-2006 (122 locations). Distances ranged from "in field" situations up to more than 2000 m. The analysis showed a tight and statistical significant correlation be-tween pollen deposition and the distance to the nearest maize field. Regression analysis showed that even in a distance of 300 m 5,3 pollen grains /cm2 can be expected (upper limit of 90% CI = 32 pollen/cm2). Pollen deposition in surrounding areas therefore must be considered to be substan-tially higher than previously assumed. It is noteworthy that, in contrast to Bt-sprays, Bt-protein in pollen seems to be unaffected by UV radiation (Ohlfest et al. 2002). With regard to soil the exposure analysis should include both Bt-protein bound to soil and Bt-protein in plant residues. Several studies show a long persistence (> 200d) of Bt-protein in soil (Tapp & Stotzky 1998; Crecchio & Stotzky 1998, 2001). Trials looking at the degradation of Bt-maize in the field under temperate climate conditions (Zwahlen et al. 2003; trials in Switzerland) also demonstrated a persistence of the Bt-protein in the soil over a period of 200 days. Similar re-sults were presented by Baumgarte & Tebbe (2005) or Hopkins &	See section 6.1.2.2 of the scientific opinion The GMO Panel "*does not consider pollen dispersal and consequent cross-pollination as environmental hazards in themselves, and is primarily concerned with assessing the environmental consequences of transgene flow on ecosystems by considering the fitness of hybrids and backcross progeny as well as exposure to non target organisms*". See section 6.1.6.1 (persistence of Bt-proteins in soil) of the scientific opinion. See section 6.1.6.2 (microbiological effects in soil) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Gregorich (2004) in Germany and Canada respectively. Zwahlen et al. (2003) indicate that ploughing can substantially slow down the release of Bt-protein and that no degradation of Bt-protein occurs during winter. The ap-plicant is asked to provide field studies over several years under different European environments regarding the persistence of the Bt toxin in the soil. Aquatic ecosystems receive Bt via runoff material from agricultural fields, detritus and by pollen deposition. Recent findings demonstrate that water and sediment can carry considerable amounts of Bt-toxin due to the cultivation of Bt-maize (Douville et al. 2007; Rosi-Marshall et al. 2007).	See section 6.1.4.5 (effects on non-target water-dwelling organisms) of the scientific opinion.
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Consideration of rare and endangered and/or protected species and protected areas The assessment of potential adverse effects on rare or endangered non-target species is of high importance for several reasons. Pollen will be transported from Bt-maize fields into the surrounding areas. In this context findings of the pollen monitoring (pollen deposition) in Germany should be taken into account (see exposure). Data from the pollen monitoring suggest that the average pollen deposition from maize fields can be expected to be 5 pollen/cm2 (total pollen shed, even distribu-tion of pollen) in a distance of 340m. Consumption of leaf area differs between butterfly species but can be considered to be one or two orders of magnitudes higher than a single cm2. Because sub-lethal effects in Lepidoptera have been recorded after the single exposure with only 5 pollen grains (Felke et al. 2002; Felke & Langenbruch 2005; Lang & Voijtech 2006) potential effects of Bt-pollen on non-target Lepidoptera should be considered in	Exposure assessment for some non target Lepidoptera species, including protected species, has been conducted by the GMO Panel based on a simulation model (see paragraph 6.1.4.2.(b)) Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			the range of several hundred meters distance from maize fields. In the context of endangered species this is of special importance since in the richly structured landscapes of Europe agricultural land is in close proximity or part of nature con-servation sites or ecologically sensitive areas (Lang 2004). Potential risks to protected species should particularly be assessed, for non-target Lepidoptera and Trichoptera (see Rosi-Marshall et al. 2007) which are in close taxonomic relationship to the target species. However, apart from one study with the monarch butterfly, no studies with relevant spe-cies have been carried out. Germany lists 87 endangered species of Macro-Lepidopteran, and 121 endangered species of caddisflies (Binot et al. 1998; numbers cited here base on the categories: critically endangered, endangered, and vulnerable). A preliminary analysis of the exposure of the German macrolepidopteran fauna showed that seven percent (97 species) of the total German macrolepidopteran species mainly occur in arable land and are potentially exposed to Bt-maize pollen. The study showed that about 39% of these 97 spe-cies are rare or endangered (Schmitz et al. 2003). The applicant is asked to thoroughly assess potential risks for rare and endangered species and to submit studies with relevant test organisms. The risk assessment should consider the potential of negative effects on protected areas. Binot, M., Bless, R., Boye, P., Gruttke, H. & Pretscher P. (1998) Rote Liste gefährdeter Tiere Deutschlands. Schriftenreihe für Landschaftspflege und Naturschutz; Heft 55. Federal Agency for Nature Conservation, Germany. Felke,M., Lorenz,N. & Langenbruch,G.A. (2002) Laboratory studies on the effects of pollen from Bt-maize on larvae of	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			some butterfly species. Journal of Applied Entomology, 126, 320-325. Felke, M., Langenbruch, G.-A. (2005) Auswirkungen des Pollens von transgenem Bt-Mais auf ausgewählte Schmetterlingslarven. BfN-Skripten 157; http://www.bfn.de/09/090203.htm#gentechnik Lang,A. & Vojtech,E. (2006) The effects of pollen consumption of transgenic Bt maize on the common swal-lowtail, Papilio machaon L. (Lepidoptera, Papilionidae). Basic and Applied Ecology, 7, 296-306. Rosi-Marshall,E.J., Tank,L.J., Royer,T.V., Whiles,M.R., Evans-White,M., Chambers,C., Griffiths,N.A., Pokel-sek,J. & Stephen,M.L. (2007): Toxins in transgenic crop byproducts may affect headwater stream eco-systems. Proceedings of the National Academy of Science USA, 104, 16204-16208. Schmitz,G., Bartsch,D. & Pretscher,P. (2003) Selection of relevant non-target herbivores for monitoring the environmental effects of Bt maize pollen. Environmental Biosafety Research, 2, 117-132.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Water organisms Potential effects of Bt-maize on aquatic organisms have so far been neglected despite the fact that aquatic ecosystems receive Bt from transgenic plants both via runoff material from agricultural fields, plant debris, and via pollen deposition. Two recent publications now indicate potential risks to aquatic non-target organisms and associated food webs. Despite of the close taxonomic relationship between Trichoptera and Lepidopera the former have never been included in the test strategies assessing Cry1Ab. Rosi-Marshall et al. (2007) demon-strated in their experiments that caddisfly larvae can be exposed to Bt when growing transgenic maize. The study also clearly demonstrated the sensitivity of caddisfly larvae	See section 6.1.4.5 (Effects on non-target water-dwelling organisms) of the scientific opinion. See also minutes of the 37th Plenary meeting of the GMO Panel where the publication of Rosie-Marshall (2007) was assessed and reported (http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/GMO_Minutes_37th_plenmeet.pdf?ssbinary=true)

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			(higher mortality and increased developmental times up to 50%) towards Cry1Ab when exposed to Bt-pollen densities in the same order of magnitude as in the field. Trichoptera, which are part of most aquatic ecosys-tems, play a major role in aquatic food webs and can be found in most inshore waters. Apart from Diptera and Coleoptera, Trichoptera represent the most diverse taxonomic group of water insects in Germany (300 species). A second recent publication indicates that Daphnia may be susceptible to Cry1Ab. Bøhn et al. (2008) observed a reduced fitness performance in combination with an earlier onset of reproduc-tion in Daphnia magna, which were fed with MON810 maize kernels ground to a particle size which can be filtered by D. magna. The authors conclude that the toxicity observed indicate a toxic effect rather than lower nutritional value of MON810 maize compared to its isoline. The eco-toxicity test with Daphnia submitted with the application (Graves & Swigert 1997; WL-96-322) cannot be con-sidered as valid because of its exposure route (maize pollen is of a larger diameter than food parti-cles used by D. magna) and test duration (the 48 h test duration does not allow to detect effects because of the mode of action of Bt). Moreover, the test was carried out with Bt11-maize. The Federal Agency for Nature Conservation urgently advise to carry out further studies assessing transport, input, and fate of Cry-Proteins from crop-byproduct into European aquatic ecosystems. The applicant is further asked to carry out experiments determining the sensitivity of caddisfly lar-vae and other makro-zoobenthos towards Cry1Ab (Pollen and plant debris). We recommend fol-lowing the experimental idea of Rosi-Marshall et al. (2007) to test caddisflies using different fee-	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			ding strategies. Bøhn,T., Primicerio,R., Hessen,D.O. & Traavik,T. (2008) Reduced Fitness of Daphnia magna Fed a Bt-Transgenic Maize Variety. Arch Environ Contam Toxicol, currently online (DOI 10.1007/s00244-008-9150-5). Rosi-Marshall,E.J., Tank,L.J., Royer,T.V., Whiles,M.R., Evans-White,M., Chambers,C., Griffiths,N.A., Pokel-sek,J. & Stephen,M.L. (2007): Toxins in transgenic crop byproducts may affect headwater stream eco-systems. Proceedings of the National Academy of Science USA, 104, 16204-16208.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Soil organisms With regard to effects of MON810 on non-target organisms in soil, the applicant did not cover the entire range of new published literature (see also Icoz & Stotzky 2008 for a review) including most relevant studies of the EU project ECOGEN (Krogh & Griffiths 2007), which integrated different levels of ecological and experimental complexity to a holistic consideration of potential effect of Bt maize (Birch et al. 2007). Although the sum of these trials suggest no or minor adverse effects of MON810 maize on different groups of soil biota (earthworms, springtails, woodlice), authors have stressed that, due to the complexity of the soil system, long-term studies and multi-species ex-periments still need to be conducted to gain an overall picture (e.g. Heckmann et al. 2006, Vercesi et al. 2006). Moreover, most studies investigated the parameters mortality and weight/growth. Ad-ditional studies arenecessary for most of the groups to include, systematically, other sublethal ef-fects and effects on behaviour. Effects on one important group of soil-inhabiting arthropods, namely mites, have still been addressed insufficiently, while some indications for effects on Diptera with soil-inhibiting larvae should be investigated further (Büchs et al.	See section 6.1.6.1 (persistence of Bt-proteins in soil) of the scientific opinion. See section 6.1.6.2 (microbiological effects in soil) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			2004). Field studies with mi-croarthropods should consider soil properties more rigorously as a shaping factor of observed ef-fects (Cortet et al. 2007). Studies addressing the communities of nematodes and soil micro-fauna and -flora still draw a contradicting picture (Griffiths 2005, 2006, 2007). As these communities are made up by a large number of different taxonomic and ecological groups, these groups should be addressed individually in future studies with regard to toxin and GMO effects. Birch, A.N.E., et al. (2007) Pedobiologia 51, 251–260. Büchs, W., Pretscher, S., and Müller, A. (2004) Auswirkungen von Bt-Mais auf terrikole, saprophage Dipte-ren-Larven, Schlussbericht Teilprojekt 1.1.4 (Förderkennzeichen 0 31 26 31 G) des Verbundprojekts: Si-cherheitsforschung und Monitoring-Methoden zum Anbau von Bt-Mais, 1-33 Cortet, J., et al. (2007) Pedobiologia 51, 207–218. Griffiths, B.S., et al. (2005) Plant Soil 275, 135–146. Griffiths, B.S., et al. (2006) J. Environ. Qual. 35, 734–741. Griffiths, B.S., et al. (2007) Plant Biotechnol. J. 5, 60–68. Heckmann, L.H., et al. (2006) Environmental Pollution 142, 212–216. Icoz,I. & Stotzky,G. (2008) Soil Biology & Biochemistry, 40, 559-586. Krogh, P.H., Griffiths, B.S. (2007) ECOGEN–Soil ecological and economic evaluation of genetically modified crops. Pedobiologia 51 171-173. Vercesi, M.L., et al. (2006) Applied Soil Ecology 32, 180–187.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Lepidoptera (part 2) Non-target effects of Bt-maize on populations of macro-Lepidoptera in the field are presently poorly understood. While a number of field studies could not observe drastic effects on butterfly popula-tions several authors point out, that the statistical power to observe changes in butterfly numbers or species	Exposure assessment for some non target Lepidoptera species, more common in European environments, has been conducted by the GMO Panel based on a simulation model (see paragraph 2.2.4.2. (b)) Additional information has been requested to the

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			assemblages are rarely met in field experiments (Lang 2004; Dolezel et al. 2005; Pra-sifka et al. 2005; Marvier 2007; Prasifka et al. 2008). Marvier et al. (2007) concluded in their meta-analysis on non-target effects of Bt crops that there are insufficient data to test for non-target ef-fects of Cry1Ab maize. However, she could show that non-target Lepidoptera were significantly reduced in Cry1Ac cotton. The difficulties to analyze small scale plots was also demonstrated by Gathmann et al. (2006) who found that only two out of 15 lepidopteran species recurrent in weed strips in maize were abundant enough to allow an analysis (3 year field experiment). In summary the information available contradicts the risk assessment of the applicant who came to the conclusion that there is no evidence demonstrating an adverse effect on organisms other than target Lepidoptera. The risk assessment of the Monarch butterfly is not suitable to assess risk for the European butterfly fauna. Dively, G.P., et al. (2004) Environ. Entomol. 33:1116-1125 Dolezel, M., et al. (2005) Ökologische Effekte von gentechnisch verändertem Mais mit Insektenresistenz und/oder Herbizidresistenz. "Rote Reihe" des Bundesministeriums für Gesundheit und Frauen - Sektion IV Band 6/05 Felke,M. & Langenbruch,G.A. (2001) Gesunde Pflanzen, 53, 24-28. Felke,M., et al. (2002) Journal of Applied Entomology, 126, 320-325. Felke, M., Langenbruch, G.-A. (2003) Gesunde Pflanzen 55, 1-7. Felke, M., Langenbruch, G.-A. (2005) Auswirkungen des Pollens von transgenem Bt-Mais auf ausgewählte Schmetterlingslarven. BfN-Skripten 157; http://www.bfn.de/09/090203.htm#gentechnik Gathmann,A., et al. (2006) Molecular Biology, 15, 2677-2685. Hansen,L.C.J. & Obrycki,J.J.	applicant by the Spanish Competent Authority and the environmental working group in relation to the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			(2000) Oecologia, 125, 241-248. Hellmich, R.L., et al. (2001) PNAS 98:11925-11930 Hofmann, F. (2007) Kurzgutachten zur Abschätzung der Maispollendeposition in Relation zur Entfernung von Maispollenquellen mittels technischem Pollensammler PMF (Estimation of maize-pollen deposition with the pollen mass filter (PMF) in relation to the distance from pollen sources. (unpublished report; Fe-deral Agency for Nature Conservation, Germany). Lang,A. (2004) Environmental Biosafety Research, 3, 55-66. Lang,A. & Vojtech,E. (2006) Basic and Applied Ecology, 7, 296-306. Losey, J.E., Rayor, L.S. & Carter, M.E. (1999) Nature, 399, 214. Marvier,M., et al. (2007) Science, 316, 1475-1477. Mattila,H.R., et al. (2005) Entomologia Experimentalis et Applicata, 116, 31-41. Prasifka,J.R., et al. (2005) Environmental Entomology, 34, 1181-1192. Prasifka,J.R., et al. (2008) Environ Entomol, 37, 1-10. Schmitz,G., et al. (2003) Environmental Biosafety Research, 2, 117-132. Traxler, A., et al. (2005) Biodiversitäts-Hotspots der Agrarlandschaft als Eckpfeiler für Risikoabschätzung und Monitoring von GVO, Bundesministerium für Gesundheit und Frauen, Sektion IV Wien, Forschungs-berichte der Sektion IV, Band 5/2005, 1-184 Zangerl,A.R., et al. (2001) PNAS, 98, 11908-11912.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Lepidoptera (part 1) The Cry1Ab toxin expressed in MON810 maize is considered to possess a relatively high specific-ity including the target organism Ostrinia nubilalis (Lepidoptera). However, the impression given by the applicant that MON810 is specific only for the target pest is not appropriate since Cry1Ab has been shown to affect many other butterfly species. A risk must be assumed since both sensitivity and exposure	Exposure assessment for some non target Lepidoptera species with different sensitivities to Cry toxins, has been conducted by the GMO Panel based on a simulation model (see paragraph 6.1.4.2. (b)) Additional information has been requested to the applicant by the Spanish Competent Authority and the environmental working group in relation to the interaction between the GM plant and TOs and NTOs.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			of Lepidoptera to Cry1Ab are likely when cultivating Bt-maize (see below). The Bt-protein will be carried via pollen up to more than 2 km into the surrounding landscape (Hofmann 2007; see also exposure). Maize pollen will settle on the host plants of Lepidoptera out-side the field. Subsequently larvae may consume corn pollen when feeding on the leaves of their host plants. Overlap of maize growing regions and butterfly habitats as well as overlap between larval butterfly stages have been demonstrated for Germany and Austria (Schmitz et al. 2003; Traxler et al. 2005) and can be expected in most if not all MS. The sensitivity of non-target lepidoptera to Cry1-toxins is well documented for a number of species (Losey et al. 1999; Hansen-Jesse & Obrycki 2000; Hellmich et al. 2001; Zangerl et al. 2001; Felke et al. 2002; Dively et al.2004; Mattila et al. 2005; Lang & Voitech 2006). Although MON810 maize pollen seems to have a low Cry1Ab content effects on non-target Lepidoptera have been demon-strated (Dively et al. 2004). Furthermore experiments from the German Federal Biological Re-search Centre for Agriculture and Forestry (now Julius Kuehn Institute) show that a single ingestion of 5-10 pollen grains (Bt176) can lead to sublethal effects on susceptible lepidopteran larvae (Felke & Langenbruch 2001, 2003, 2005; Felke et al. 2002). The results from this working group also demonstrated the sensitivity of two butterfly species (Inachis io and Aglais urticae) living in habitats adjacent to maize field which may be used as a model for other – including protected – butterfly species. Sensitivity data (Cry1Ab) also exist for the European swallowtail Papilion machaon. Lang & Voitech (2006) demonstrated that this species is highly sensitive to Bt pollen (Bt176). Their ex-periments showed that an	See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			average consumption of only 9.9 pollen would kill 30% of the larvae after 14 days. Moreover, LD30/LC50 were found to be approximately two times lower than LD50/LC50 values and exposure to Bt pollen also affected other fitness related parameters of adult butterflies such as wingspan (Lang & Voijtech 2006). Sensitivity data on micro-lepidoptera and moth are virtually missing. However even more distinct taxonomic groups such as caddisflies, a taxonomic group close to the Lepidoptera, were found to be sensitive to Bt-pollen (Cry1Ab) indicating the urgent need to test moth species and micro-lepidoptera.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	Epigeic organisms MON810 maize has been in the focus of numerous field studies since 1998 including research in Germany and other parts of Europe. Most of the studies on the epigeic arthropod fauna in maize fields did not show major negative effects of the cultivation of MON810 (e.g. Bruck et al. 2006; Dale et al. 2005; Eckert 2006; Eckert et al. 2006; Lang et al. 2005; Ludy & Lang 2006; Meissle & Lang 2005; Rauschen et al. 2004; Rezac et al. 2006; Schuphan 2003, 2005; Toschki et al. 2007). However, several authors explicitly point out that field studies may not possess the statistical po-wer to identify minor changes in the abundance or the composition of species (Marvier et al. 2007; Lang 2004; Lang et al. 2005). In a meta-analysis of available field data of Cry1Ab maize Marvier et al. (2007) concluded that the effects of Bt-maize on arthropod taxa were lower than routine insecticide applications but higher than non-GM maize varieties not treated with insecticides. Bruck,D.J., Lopez,M.D., Lewis,L.C., Prasifka,J.R. & Gunnarson,R.D. (2006) Effects of transgenic Bacillus thuringiensis corn and	See section 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			permethrin on nontarget arthropods. Journal of Agricultural and Urban Entomol-ogy, 23, 111-124. Daly,T. & Buntin,G.D. (2005) Effect of Bacillus thuringiensis transgenic corn for lepidopteran control on non-target arthropods. Environmental Entomology, 34, 1292-1301. Eckert,J. (2006) Effekte des Anbaus von Bt-Mais auf Nichtzielarthropoden der Krautschichtfauna – Monito-ringorganismen und praktikable Erfassungsmethoden. Doktor der Naturwissenschaften RWTH Aachen. Eckert,J., Schuphan,I., Hothorn,L.A. & Gathmann,A. (2006) Arthropods on maize ears for detecting impacts of Bt maize on nontarget organisms. Environmental Entomology, 35, 554-560. Lang,A. (2004) Monitoring the impact of Bt maize on butterflies in the field: estimation of required sample sizes. Environmental Biosafety Research, 3, 55-66. Lang, A., Beck, R., and Bauchhenß, J., 2005 Monitoring der Umweltwirkungen des Bt-Gens, Bayerische Landesanstalt für Landwirtschaft (LfL), Schriftenreihe, 7/2005, 1-115 Ludy,C. & Lang,A. (2006) Bt maize pollen exposure and impact on the garden spider, Araneus diadematus. Entomologia Experimentalis et Applicata, 118, 145-156. Marvier,M., McCreedy,C., Regetz,J. & Kareiva,P. (2007) A Meta-Analysis of Effects of Bt Cotton and Maize on Nontarget Invertebrates. Science, 316, 1475-1477. Meissle,M. & Lang,A. (2005) Comparing methods to evaluate the effects of Bt maize and insecticide on spi-der assemblages. Agriculture Ecosystems & Environment, 107, 359-370. Rauschen,S., Eckert,J., Gathmann,A. & Schuphan,I. (2004) Impact of growing Bt-maize on cicadas: Diver-sity, abundance and methods. IOBC wprs Bulletin, 27, 137-142. Rezac,M., Pekar,S. & Kocourek,F. (2006) Effect of Bt-maize on epigeic	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			spiders (Araneae) and harvestmen (opiliones). Plant Protect.Science, 42, 1-8. Schuphan, I., (2003) Verbundprojekt: Monitoring der ökologischen Auswirkungen insektenresistenter Kultur-pflanzen mit rekombinanten Bacillus thuringiensis Toxin-Genen - Teilprojekt 1: Auswirkungen auf Insek-tenpopulationen im Agrarbereich, Technische Hochschule Aachen; project no. 0312175 Schuphan, I., (2005) Effekte des Anbaus von Bt-Mais auf die epigäische und die Krautschichtfauna ver-schiedener trophischer Bezüge, project no. 0312631c Toschki,A., Hothorn,L.A. & Ross-Nikoll,A. (2007) Effects of cultivation genetically modified Bt maize on epi-geic arthropods (Araneae; Carabidae). Environmental Entomology.	
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 09 Mechanism of interaction between the GM plant and target organisms (if…	The applicant conducted 8 studies to assess potential risk of MON810 to NTO which were elaborated between 1992 and 1997. These studies do not represent the state-of-the-art regarding testing of NTO. However, a huge number of lab, semi-field and field studies were conducted since the first market introduction of MON810, which addressed potential impact of MON810 on NTO. The applicant listed relevant peer reviewed publications. Controversial aspects such as potential effects on Lepidoptera or lacewings were discussed in more detail. Regarding potential effects on soil functions or degradation processes of Cry1Ab not all relevant publications are taken into consideration. It is desirable that the applicant discusses these aspects in more detail. Considering all given information the German CA concludes that potential adverse impact of MON810 on NTO is negligible.	(-)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 12.01 General	The monitoring plan presented by the applicant is in need of major revisions and should state clearly the information (e.g. open literature or industry studies) on which conclusions were drawn. The applicant should consider to make use of already established monitoring networks in member states according to Annex VII, C. 3.2. of Directive 2001/18/EC for the implementation of the monitoring plan presented. The applicant should describe all monitoring activities for MON810. A way forward could be additional implementation plans e.g. as elaborated by Monsanto in context with the temporary suspension of the authorization to distribute maize seeds MON810 for commercial planting in Germany by the German Federal Office of Consumer Protection and Food Safety (BVL) of 27. April 2007. We want to point out that, if a reasoned suspicion of an adverse effect evolves, the EU and the Member States have to be informed immediately.	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (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.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	Interplay between environmental risk assessment and monitoring Additional Comments of the Federal Agency for Nature Conservation (BfN): In reference to our detailed comments on the data provided by the applicant (see D:9.) the Federal Agency for Nature Conservation is of the opinion that a case specific monitoring of MON810 is essential. To ensure that anticipated, unanticipated as well as effects that are difficult to predict are covered by the monitoring plan, the borderline between CSM and GS should be flexibly handled. Particularly effects, that are difficult to predict can either fall under GS, CSM or both simultaneously. Thus in the following some relevant monitoring parameters are listed under CSM (D.11.3.) as well as under GS (D. 114.).	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC"*. *"The EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel also recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				surveillance through farm questionnaires".
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	Comments of the Federal Agency for Nature Conservation (BfN): The applicant's proposal for an environmental monitoring plan does not fully meet the requirements according to Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC. The provided monitoring plan is very general and needs further specification and amendment. The Federal Agency for Nature Conservation is of the opinion that a detailed and meaningful monitoring plan has to be provided before consent can be given. The aims of environmental monitoring of MON810 are to serve as an early warning system: "The data which will be monitored should be relevant to and suitable for a rapid assessment and implementation of measures to reduce any consequences to the environment." (Council Decision 2002/811/EC). In order to assess, whether the monitoring plan is appropriate to fulfil this task, a provision of a specified list of monitoring parameters is needed. The applicant is requested to present for each parameter a detailed statement of the parameter definition, the observation methods (collection and analysis of samples with references), the frequencies of observations (time and number of visits to collect data) and the monitoring locations including number and size. Furthermore, an operating schedule giving full details of points in time as well as elaboration of the methods of data analysis including the statistical methods is requested. To ensure the compliance with fundamental quality criteria and the comparability of monitoring data from different regions and EU Member States, standard	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant and advises that the potential evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance in pest populations.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			methodology should be followed where appropriate (e.g. CEN, OECD-Methods or VDI Guidelines). In case of monitoring data being collected by external persons or institutions other than the applicant, binding agreements/contracts with third parties are requested which clearly determine which data will be provided and how these data will be made available. The time-period of monitoring needs to be sufficient to detect delayed, long-term or cumulative adverse effects. Therefore, it may be necessary to extend the monitoring of certain parameters beyond the period of the consent. According to Directive 2001/18/EC the responsibility for the monitoring plan is on the applicant. Any shifting of responsibilities to the public or to competent authorities as proposed by the applicant in chapter 11.4.2.3 and 11.4.3.1.2 of the application should be declined.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.02 Case-specific GM plant monitoring	Comments of the Federal Agency for Nature Conservation (BfN): Due to incomplete data and high uncertainties concerning the risk assessment of MON810 the applicant is requested to provide a case-specific monitoring plan including the following monitoring objects: • Monitoring of MON810 volunteers • Monitoring the exposure of Bt Toxin to the environment (field, surrounding areas, including semi natural and natural areas) via pollen or plant residues o pollen monitoring o persistence and accumulation of Bt-toxin in soil, water and sediments • Monitoring the impact of MON810 on non target organisms (relevant indicators have to be defined) o Lepidoptera o aquatic organisms o soil organisms o pollinators or pollen feeding insects o phytophages, predators and parasitoids • Monitoring the abundance and damage caused by secondary pests	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC*". *"The EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel also recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires".*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 12.02 Case-specific GM plant monitoring	The applicant proposes an insect resistance management plan to prevent Bt-resistance of European or Mediterranean corn borers (Appendix V). However, according to the relevant EFSA guidance document measures for early detection of resistance development should be regarded as the case specific monitoring.	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC"*.
Germany	Federal Office of Consumer Protection and Food Safety (BVL)	D, 12.03 General Surveillance of the impact of the GM plant	The applicant will use the stewardship program, peer reviewed publications, existing surveillance systems and farm questionnaires for general surveillance. According to the EFSA guidance document an important task within general surveillance is to link monitoring to protection goals. The applicant should provide more information regarding existing of networks already established in the different countries which can be used for monitoring purposes. In this context, the applicant should be aware of those networks comprehending data on effects on biodiversity and on the food chain, and should describe the generic methods how to approach these existing networks. The applicant should lay down the steps for identifying established local and regional surveillance systems and their priorities and their application for monitoring impacts of the GM maize. Furthermore, the applicant should include a description how to evaluate and select existing surveillance systems which are already monitoring one or more of the relevant goals. Farm questionnaires are a suitable method for general surveillance in agronomic environment. However, some questions e.g. in section 2 access the efficiency of product quality, labelling or farmer training	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			are to be optimised for detecting unforeseen adverse effects on the environment or defined protection goals. The questionnaire can be improved. The applicant has a legal obligation to report regularly about the monitoring activities. A full analysis and evaluation of all data is in the responsibility of the applicant. The Competent Authorities are not in charge of collation and evaluation of data of existing networks as described in chapter 11.4.3.2.2. and shown in fig 22. It is recommended to report annually on the monitoring activities and data should be regularly analysed and evaluated e.g. every third year.	The GMO Panel is satisfied with the reporting interval proposed by the applicant. See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	e) According to Council Decision 2002/811/EC the "determination of the baseline status of the re-ceiving environment is a pre-requisite for the identification and evaluation of changes observed via monitoring." In the context of baseline data the applicant mentioned historical knowledge and ex-perience of users of MON810 only. The Federal Agency for Nature Conservation is of the opinion that in addition a scientifically sound collection of baseline data is needed. Therefore the applicant is requested to: • explain, how scientifically sound baseline data will be provided • take into account that baselines may differ depending on geographical regions, • consider that the time period and the replication of sufficient baseline observations will de-pend on the specific monitoring parameter, • implement direct comparison with non GM-plant reference areas if appropriate. f) According to the monitoring plan provided by the applicant it is foreseen to actively monitor exist-ing information sources such as official websites, scientific publications and expert reports on GMOs in order to identify, collate and follow up on potentially adverse observations made for MON810	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			(chapter11.4.3.2.4 of the application). The monitoring report on MON810 (2005) already provided a compilation of numerous publications. However, these publications are neither sys-tematised nor analysed with respect to the risk assessment. Therefore the applicant is requested to: • specify, how additional information sources will be evaluated and analysed, • explain, how the results of the analysis will be presented.	*(…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	General surveillance of the impact of the GM plant a) As stated by the applicant, farm questionnaires are the key element of the general surveillance plan of MON810. The farm questionnaires presented consider almost exclusively qualitative data on agronomic issues on field. Therefore they might provide useful feedback to the consent holder for commercial and development purposes. However, they are not appropriate to monitor environ-mental effects on farm level respectively in the surrounding of MON810 fields. Neither relevant environmental parameters are addressed nor quantitative, high quality data are included thus sci-entifically sound analyses are not possible. Therefore, with respect to potential environmental ef-fects, additional systematic and scientifically sound monitoring tools are needed. b) The applicant stated that a range of monitoring parameters has been derived and a range of influencing factors to be monitored has been identified additionally (see 11.4.3.2 of the application); further on that these parameters can be associated with desirable protection goals (listed in chap-ter 11.4.2 of the application). However, in the provided monitoring plan for MON810 no monitoring parameters or influencing factors are specified. As mentioned above, a specified list of monitoring parameters for	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			general surveillance is needed. The applicant is requested to provide a detailed general surveillance plan including the following monitoring objects: • Monitoring of Bt-maize outside fields (ferals) • Monitoring the exposure of Bt-Toxin on the environment via pollen or plant residues (field, surrounding areas, including semi natural and natural areas) o pollen monitoring o persistence and accumulation of Bt-toxin in soil, water and sediments • Monitoring the impact of MON810 on non target organisms (relevant indicators have to be defined) o Lepidoptera o aquatic organisms o soil organisms o pollinators or pollen feeding insects o phytophages, predators and parasitoids o birds o mammals o different levels of food chain • Monitoring changes in diversity of habitats, biota and landscape structure • Monitoring of impacts on soil functions c) It is stated by the applicant that the information of selected existing observation networks will be additionally used for general surveillance of MON810. However, there is no specification which existing networks will be included nor what data will be used and how these data will be made available. Therefore the applicant is requested to: • specify which parameters will be monitored through existing monitoring programs, • specify the criteria and detailed approach used to evaluate existing monitoring programs and how appropriate programs will be selected, • describe how arrangements for collecting, collating and analysing data will be made. d) According to the applicant, the introduction of MON810 is not confined to specific geographical zones and monitoring activities are mainly focused on areas where MON810 has a high marked penetration. However, the application does not specify how relevant monitoring sites and areas will be	improve the PMEM proposed by the applicant.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
			selected and which scientific criteria will be considered. Therefore, the applicant is requested to: • specify the criteria used to select appropriate monitoring sites and areas, • consider the biogeographic variation, the wide variety of different climatic conditions, the different land use forms and management practices within Europe, • provide a spatial monitoring design that is sufficient to support statistical analysis of results based on good scientific practice.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.06 Reporting the results of monitoring	Comments of the Federal Agency for Nature Conservation (BfN): The monitoring results have to be reported on an annual basis. All raw data have to be provided upon request. The applicant should make use of the monitoring format provided by the Commission and agreed on by Member States. According to Directive 2001/18/EC (Art.20 point 4) the results of the monitoring carried out under part C of the Directive shall be made publicly available. Therefore the applicant is requested to state, how the monitoring results will be published.	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 (…).*
Hungary	Ministry of Environment and Water	General comments	Sampling and detection: The application shall be accompanied by the methods for detection, sampling (including references to existing official or standardised sampling methods) and identification of the transformation event and, where applicable, for the detection and identification of the transformation event in the food and/or in foods from it or in the feed and/or in the feed produced from it (Articles 5 (3) i) and 17 (3) i) of Regulation 1829/2003/EC). It is known that sampling methods determine the detection limit and the measurement uncertainty. There is no guideline in the	This point is outside the remit of the GMO Panel.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			notification regarding this particular issue. The Community Reference Laboratory of the European Union issued a guideline on the minimum performance requirements for analytical methods of GMO testing (http://gmo-crl.jrc.it/doc/Method%20requirements.pdf). As a consequence, the sampling method should ensure the appropriate detection and quantification which is – taking into account the 0,9 % threshold level – at least LOD: 0,045% and LOQ: 0,09%. Taking into account that the above mentioned Regulation refers on the detection and identification of the transformation event not only in the plant (maize) but also in the foods/feeds produced from it, an appropriate sampling method should be provided also for some typical maize containing food and feed mixtures. This sampling method should be of appropriate sensitiveness and enable the adequate quantification. The notifier states that „The protein present in MON 810 can also be detected by an appropriate ELISA method." This statement is quite imprecise regarding the ELISA method which can be applied. It is not clear whether such methods has been elaborated, which proteins (or which regions of the protein) can be detected by these methods, what is the specificity and sensitiveness, etc. of the method, where the relevant documentation can be found. The notifier mentions the event-specific method and refers on the draft ISO 21570 standard. This standard is in force years ago. It is not clear whether the method complies with the above mentioned minimum performance requirements, taking into account that the LOD and the LOQ is only "at least" 0,1%. (As we know, the validated and standardized methods can be more or less sensitive and therefore appropriate or not appropriate in this regard. Compared to the	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			above mentioned performance criteria this method seems not to be appropriate.) The specificity of the above mentioned method was tested in 14th of September 2004. on Event176, Bt11, T25, GA21, GTS 40-3-2 GMO events. From that time several new GMOs were authorized, or are under authorization in the EC. It is necessary to test the specificity of the above mentioned method on these new GMOs. Has the notifier provided any kind of evidence, whether the gene construct, the place and environment of the insertion in the sample sent to the Reference Laboratory of the EC in 1999 and in current marketed MON810 hybrids has changed or not? How will be ensured that the Institute for Reference Materials and Measurements (IRMM) receives the necessary MON810 material in order to be able to provide certified reference materials to the enforcement laboratories?	
Hungary	Ministry of Environment and Water	General comments	Potential adverse effects of MON810 in the different biogeographical regions in the EU: Taking into account that there is a great regional variation in species composition and abundance, and agricultural practices in Europe significantly diverge, potential effects of the genetically modified MON810 maize line on non-target organisms depend on geographical factors. Therefore, we believe that all of the different biogeographical regions within the EU – including the Pannonian – should be taken into account in the environmental risk assessment of MON810 which can not be found in the Monsanto documentation. In our opinion, there is a need on in-depth analysis in this regard. Also studies assessing the potential effects on Lepidoptera should be based on species commonly found in the EU, not other regions of the World. Long term effects of MON810: A thorough assessment of	See EFSA guidance document (2006) "*data should be provided from field experiments in areas representatives of those geographical regions where the GM plant will be grown commercially in order to reflect relevant meteorological, soil and agronomic conditions*". See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902002009.htm). See sections 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			potential long term toxicological and allergological effects are also missing from the documentation. Therefore, further information is needed in this regard based on relevant scientific studies.	
Hungary	Ministry of Environment and Water	General comments	During the risk assessment, following articles should also be assessed: The Bt toxin directly and specifically binds glycolipids. This binding is carbohydrate-dependent and relevant for toxin action in vivo (J.S. Griffitts, S.M. Haslam, T. Yang, S.F. Garczynski, B. Mulloy, H. Morris, P.S. Cremer, A. Dell, M.J. Adang and R.V. Aroian: Glycolipids as receptors for Bacillus thuringiensis crystal toxin. Science 307, 922-925 (2005). With the work of Vazquez-Padron and others, however, it has been demonstrated that Bt toxins bind not only to the insect gut but also to the mammalian gut, leading to various immunity problems. (Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.K., Doerfler, D.L. and Seligy, V.L. (1999). Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides (Environmental Health Perspectives 107, 575-582). The claimed exclusiveness of the specificity of Bt toxin-binding to the insect gut can therefore no longer be maintained, as there is credible scientific evidence that some Bt toxins will also bind to the gut of mammalian species (A. Pusztai and S. Bardocz: GMO in animal nutrition: potential benefits and risks. In: "Biology of Nutrition in Growing Animals" (ed. Mosenthin, R. Zentek, J.and Zebrowska, T.)	(-)

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			2006 Elsevier Limited, pp. 513-540). The capacity of various A-B toxin-lectins, including Bacillus thuringiensis (Bt) Cry1Ac protoxin to stimulate and modulate both the systemic and mucosal immune systems is now firmly established (RI. Vázquez, L. Moreno-Fierros, L. Neri-Bazán, G.A. De la Riva and R. López-Revilla: Bacillus thuringiensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scandinavian Journal of Immunology 49, 578-584 (1999); Vazquez Padron, R.I., Moreno Fierros, L., Neri Bazan, L., De la Riva, G.A. and Lopez Revilla, R. Intragastric and intraperitoneal administration of Cry1Ac protoxin from Bacillus thuringiensis induces systemic and mucosal antibody responses in mice. Life Sciences 64, 1897-1912. (1999); Vazquez-Padron, R.I., Moreno-Fierros, L., Neri-Bazan, L., Martinez-Gil, A.F., de la Riva, G.A. and Lopez-Revilla, R. Characterization of the mucosal and sytemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Brazilian Journal of Medical and Biological Research 33, 147-155 (2000); Vazquez Padron, R.I., Gonzalez Cabrera, J., Garcia Tovar, C., Neri Bazan, L., Lopez Revilla, R., Hernandez, M., Morena Fierros, L. and De la Riva, G.A. Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochemical and Biophysical Research Communications 271, 54-58 (2000). In a more recent study the cellular immune response induced by Cry1Ac and its mutants in mice has been analysed (G.G. Guerrero, W.M. Russel and L. Moreno-Fierros: Analysis of the cellular immune response induced by Bacillus thuringiensis Cry1Ac toxins in mice: Effect of the hydrophobic motif from diphtheria toxin. Molecular Immunology 44, 1209-1217 (2007). It was shown that the production of Th1 and Th2 type cytokines by	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Cry1Ac toxins was inhibited by N-acetylgalactosamine, in accordance with the lectinic properties of this Bt toxin. Effect of Bt toxin on human cells: Tayabali AF and Seligy VL. Human cell exposure assays of Bacillus thuringiensis commercial insecticides: production of Bacillus cereus-like cytolytic effects from outgrowth of spores. Environ Health Perspect 108: 919-930, (2000).	
Hungary	Ministry of Environment and Water	D, 03 Information on the expression of the insert	The notifier refers to an EFSA opinion. In our view, the documentation has to provide the source of the results of the measurements and the notifier's assessment based on these data. Some of the data can be found in Part (b) but adequate explanation has not been provided. There is no explanation for the reason for different Cry1 toxin levels in the leaves and no information has been provided whether these data are significant. There is no explanation for the changeable expression of toxin either (see Table 5 (1994, USA), Table 6 (1995, France and Italy) and Table 7 (1995, France and Italy)). Does the nutrition supply have any impact on the expression levels of the toxin? Are expression levels of the toxin different among various MON 810 varieties? How stable (constant) is the amount of the expressed Cry1 toxin? How has the amount of the Cry1 toxin been measured in 1995 taking into account that the kit (ENVIROLOGIX) which was widely used for this purpose has been withdrawn from the market? There is no information on the amount of the expressed Cry1 toxin per hectare – that was one of the questions raised by EFSA regarding the scientific information included in the background document of the Hungarian prohibition on maize MON 810. We believe that Monsanto also has to	The original application provided sufficient data on protein expression levels. In 1994, field trials were conducted at six locations distributed throughout the major U.S. maize growing region representing a variety of environmental conditions. In 1995, five field trials were conducted within the major maize growing regions of France and Italy. With regard to Cry1Ab, the protein levels were similar for plants grown in the United States and European field trials over two consecutive generations. The levels of Cry1Ab detected did not raise any safety concerns and there is no new data to question this opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			provide these data in the technical dossier. Furthermore, data from years 1994 and 1995 provided by the Monsanto has not been assessed biometrically.	
Hungary	Ministry of Environment and Water	D, 06 Any change to the ability of the GM plant to transfer genetic material to…	The documentation does not provide relevant information or scientific data of the spontaneous DNA-absorption of bacteria which make the MON 810 silage as well as the gene transfer (conjugation) from these bacteria to bacteria living in cow's rumen.	In the extremely unlikely event that functional DNA is transferred horizontally there are no components of the GM plant which cause any concern for animals
Hungary	Ministry of Environment and Water	D, 07.01 Comparative assessment	It is clear that the chemical composition of the MON810 transgenic line cannot be substantially equivalent with the chemical composition of the parental line, taking into account the presence of theCry1Ab toxin in MON810. Therefore, the MON810 – as food or feed – cannot be as safe as the parental line and have no harmful effects on human or animal health. In the framework of the compositional analysis, MON810 transgenic maize line should be compared exclusively to the isogenic parental line, which has been grown under identical conditions and at the same time. The comparison of MON810 to other hybrid lines such as the MON818, or other transgenic lines, or commercially available hybrid lines, or to similar lines referred to in the literature cannot be scientifically justified. According to the Monsanto documentation, a non-isogenic parental line has been used as control. Also, data from Table 10 show significant differences. The amino acid	The GMO Panel considered total compositional data supplied by the applicant which have become available since the original authorization (see, Section 3.2.2) and concludes that maize MON810 is compositionally equivalent to the non-GM counterparts MON 820 and MON 818 and to other conventional maize varieties except for the presence of the Cry1Ab protein. The Panel is not aware of any new compositional data that will lead to reassessment of its previous opinions.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			composition of MON810 and the "control line" shows significant differences in alanine, cystine, histidine, phenylalanine, proline, serine, tryptophan and tyrosine contents. Likewise, the fiber and calcium contents also show significant differences. The protein content should not be compared to data of Jugenheimer (1976), since the methods used for the measurement of the protein content were entirely different 30 years ago. In our view, there are similar problems with the data originating from 1995 European field trials. In these experiments, MON820 transgenic line was used as control, which is not the isogenic line. There are significant differences in fat, methionine and tryptophan contents (see Table 11). Furthermore, we do not see the reason for the comparison of MON810 to other events containing more, and other transgenes, since they produce other compounds and different cry toxins.	
Hungary	Ministry of Environment and Water	D, 07.02 Field trials	Production of material for comparative assessment From scientific point of view we disagree with the validity of comparison of MON810 to other conventional hybrid maize varieties.	See comment above
Hungary	Ministry of Environment and Water	D, 07.03 Selection of compounds for analysis	From scientific point of view we disagree with the validity of comparison of MON810 to other conventional hybrid maize varieties.	See comment above
Hungary	Ministry of Environment and Water	D, 07.05 Product Specification	The long history of safe consumption of conventional maize does not support the conclusion that MON810 is also safe for consumption. The Cry1Ab protoxin of various Bt preparations (pesticides) is not the same as the transgenically produced Cry1Ab in the MON810 event. The longer form (protoxin) of the Bt toxin is dominant in Bt preparations, while transgenic plants contain the gene of the shorter active toxin. After spraying the plants with the pesticide, the Bt preparation can be found on the surface of the plants only. In contrast, all the cells of the GM	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization show that MON810 maize and its products are as safe to the experimental animals as traditional maize and its products.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			plant produce the shorter, active Bt toxin. Therefore, the safety of the conventional Bt preparations does not warranty the safety of the maize line MON810. The fact that MON810 has been consumed by animals and humans since 1997 does not necessarily guarantee its safety as food or feed, taking into account that the development of long-term effects might need several generation times to develop. Furthermore, in our view the food and feed safety experiments were not thoroughly carried out. The fact that no problems have been identified yet in this regard proves only that the MON810 has no immediate, acute toxicity.	
Hungary	Ministry of Environment and Water	D, 07.06 Effect of the production and processing	There are no references in the scientific literature in which the safety of Cry1Ab toxin – isolated from the GM plant has been investigated. We believe that there is a need for further information in this regard.	This comment is addressed by the GMO Panel in the paragraph 5.1.2 of the scientific opinion.
Hungary	Ministry of Environment and Water	D, 07.08 Toxicology	III. 13-week feeding study in rats: In our view, the experimental design of the feeding study is not appropriate. The animals were allowed to consume their feed ad libitum. In a well designed scientific study, the feeding has to be strictly controlled. All animals should be pair-fed (fed with the same amount of diet, protein, and calories) in order to be able to compare their weight. The comparison of the MON810 test group to a population of rats fed with diet containing non-transgenic conventional maize is interesting, but scientifically not relevant. The MON810 test group should have been compared only to the control group fed with the isogenic maize line. The energy and protein content, as well as the composition of the diets should have been the same in both cases. Detailed data on the 13-week feeding study are missing from the documentation. The mice feeding study which has been carried out by Naylor in 1992 has	The GMO Panel has concluded that the available animal studies provided by the applicant as well as the literature data that have become available since the original authorization and reviewed by the Panel in the MON810 opinion show that MON810 maize and its products are as safe to the experimental animals as traditional maize and its products. The Panel does not consider long time animal feeding studies to be necessary. Feeding studies with several target animal species (broiler chickens, lactating diary cows, Atlantic salmon) have shown that Maize 810 is nutritionally equivalent to conventional non-GM maize.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			not been published in the scientific literature and is not available via Internet. Broiler chicken feeding study: According to the data of the technical dossier, there are significant differences in the amount of the diet consumed – animals fed with MON810 maize containing diet consumed significantly more, there were differences in the weights of the breasts and thighs. The comparison of the MON810 test group to a population fed with diet containing non-transgenic conventional maize is scientifically not relevant. The MON810 test group should have been compared only to the control group fed with diet containing isogenic parental maize line. There is no reference by whom the broiler chicken study was carried out and whether it has been published. In our opinion, the referred data of the dossier are not sufficient to be able to come to a conclusion that the MON 810 maize line is safe in toxicological point of view. We believe that further information is needed in this regard.	
Hungary	Ministry of Environment and Water	D, 07.08 Toxicology	II. The in vitro digestibility experiments of the Cry1Ab protein have also been carried out with the E. coli recombinant protein, and not with the protein isolated from the transgenic plant. When analyzing the digestibility in the stomach it was found that 90% of the Cry1Ab protein was degraded within 2 minutes. In our view, these data are insufficient to come to a scientific conclusion. In the in vitro gastric digestibility experiments, simulating the processes occurring in the stomach, almost all proteins can be digested at low pH, in the presence of large enzyme quantities. An example is PHA, the bean lectin, which survives passing through the entire gut in biologically and immunologically intact form. This protein, and several other lectins resist the effects of the digestive enzymes produced by the	See section 5.1.3 of the scientific opinion

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			intestinal system and also by microbes. Under these conditions (low pH (1,2-2,0) and at high digestive enzyme-test protein ratios almost all proteins can be fully degraded in vitro. In contrast, in the human stomach pH is seldom as low as pH 1,2-2,0. The production of gastric acid in newborns, small children and elderly people is rarely gets below pH 2. A large number of adults often take acid blockers against acid overproduction, especially in case of H. pylori infection. In vitro tests therefore can seldom mimic the real in vivo conditions. It is clearly a warning sign that in the in vitro intestinal proteolysis-simulation model, in which the enzymes extracted from the bowels have been used, the Cry1Ab protein has not been degraded even after 19 hours. However, it is not surprising, taking into account the fact that the Cry1Ab protein is a lectin, and lectins are seldom degraded fully in the mammalian digestive system. No evidence has been provided in the documentation on the long-term toxicity of the product either. It is known that Cry toxins have no acute toxic effect including target insects as well. However, the chronic effect of a compound is connected with long term consumption. There can be remarkable differences. There is no information in the dossier, how many Cry-toxin gets into the cow/pig/chicken digestive system by consumption of MON 810 feed or silage. There is no information on the gene transfer to bacteria which make the MON 810 silage as well as the gene transfer from these bacteria to bacteria living in the cow's rumen. We believe that further information is needed in this regard.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Hungary	Ministry of Environment and Water	D, 07.08 Toxicology	I. We have already mentioned under "General comments" that further peer-reviewed scientific articles should be taken into account during the risk assessment (see above). The documentation refers on the EFSA opinion from 2005 and not on particular scientific data. The notifier should provide new scientific data from experiments regarding the environmental and dietetic safety of MON 810 maize which has been carried out in the last 10 years answering those questions which were raised in the meantime. The notifier states that experiments carried out with the Bacillus thuringiensis toxin do not shows chronic effects but these data are not thoroughly assessed in the documentation. We disagree with this statement. It is not proved that long term consumption of the Cry1 toxin does not cause allergy – taking into account that the immune system of digestive system stimulating effect of lectins is well known. The statement, that animals consume the GM maize long ago (under uncontrolled conditions) and without any acute effects can not be considered as scientific information. The toxin produced by the Bacillus thuringiensis is not the same produced by the MON 810 plant. The range of effect and specificity of the Cry-toxin expressed by the GM MON 810 plant differs from the bacterial Cry toxins (five in Dipel). Data resulting from studies carried out with Cry1 toxin produced by Escherichia coli should be compared to the data with Cry1 toxin extracted from MON 810 plants. To summarize, the protoxin of the Bt toxin is dominant in Bt preparations, while the transgenic plants produce a shorter version, the safety of the Bt spray used for the last 45 years does not proves the safety of MON810. It is true, that the amount of the newly expressed protein is low in the genetically modified maize. However, using affinity chromatography methods, enough material	The GMO Panel considered the information provided in the initial application evaluated by the Scientific Committee on Plants (SCP, 1998), new information supplied by the applicant, and the Member States' comments and a referenced review and discussion of the new scientific data published on maize MON810 from independent sources since the original authorisation of this maize by the European Commission (EC, 1998) provided by the applicant, when relevant for a renewal of the food/feed safety of maize MON810.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			can be obtained for the investigations, provided that the starting material is available in large quantities. This maize has been cultivated in large areas since 1997, therefore we believe that there is an opportunity to use the toxin produced by the GM plant for the digestibility studies instead of the E. coli recombinant protein in investigations regarding the degradation of the protein. It should also be taken into account that the post-synthetic modification of the recombinant protein in prokaryotes differs from that in eukaryotic cells. Therefore, safety studies should be carried out with the isolated protein produced by the GM plant. The terminal amino acid analysis is not sufficient to decide whether two proteins were the same, since iso-proteins, partially synthesized, or post-synthetically differently modified proteins can also be present. When determining the identity of molecular weights the SDS-page method itself is not sufficient. Exclusively the thermodynamic methods (ultracentrifuge), or the MALDI-TOF are only appropriate in this respect. Furthermore, Bt-protoxins which are sprayed in the framework of a conventional plant protection measure get in a remarkable lower amount on the surface of the leaves. The protoxin will only be activated in the digestive system of the insects. In contrast, in the GM plant all cells produce the active toxin. Therefore, those data which proves the safety of the pesticide are not relevant when considering the safety of MON 810. The acute toxicological studies have also been carried out with the E. coli recombinant protein. The results of those investigations do not prove that the Cry1Ab protein expressed by the GM plant is non-toxic. The documentation does not contain data on the weight of the different mouse organs, or histology, therefore, there is no evidence provided to prove the lack of toxicity of the Cry1Ab protein for mice.	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Hungary	Ministry of Environment and Water	D, 07.09 Allergenicity	It is known from the scientific literature that even the homology of 6, or sometimes 4 amino acids of an epitope can cause allergy. We have already mentioned the problems with the protein digestibility studies (stimulated gastric fluid) under Part I, D 7.8 of the technical dossier. We believe that there is a need on further information in this regard. Technical dossier, Part I, D 7.9.1. Assessment of allergenicity of the newly expressed protein: According to the scientific literature, Bt cry toxins are immunogens and immune adjuvants, and their possible allergenicity cannot be excluded either. It is not clear what is the meaning that "no biologically significant homology" was observed. Were there any sequence homologies observed in the databases? Were these considered biologically irrelevant? In our view and according to the scientific literature, protein degradation studies in stimulated gastric fluid often differ from the results of in vivo experiments (see PHA). According to the intestinal protein degradation studies the Cry1Ab protein is not degradable. Therefore, regarding the allergenicity of the Cry1Ab protein the presented data are not convincing. We believe that there is a need for further information in this regard.	See section 5.1.4 of the scientific opinion
Hungary	Ministry of Environment and Water	D, 07.10 Nutritional assessment of GM food/feed	Detailed data are missing from the dossier to prove the food and feed safety of the MON810 line. We believe that there is a need for further information in this regard.	See section 5.1.5 of the opinion
Hungary	Ministry of Environment and Water	D, 08 Post-market monitoring of GM food/feed	(Technical dossier, Part I, D 7. 11. - Post-market monitoring of MON810 food/feed) After studying to the data of the dossier we are not convinced of the safety for food and feed use of the MON810. Furthermore, no long-term toxicological studies have been carried out. No strategy has been elaborated for long-term monitoring of potential health effects.	See section 5.1.6 of the opinion

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Hungary	Ministry of Environment and Water	D, 10.04 Interactions between the GM plant and target organisms	(Technical dossier, Part I, D 9. 4. - Effects on target organisms) The documentation assesses under section D, point 9.4 the interactions between MON 810 and Ostrinia nubilalis as well as Sesamia nonagroides as main target organisms. These Lepidopteran pests of the maize are characteristic for the South-European region but not for the Hungarian agro-ecosystem. Potential effects on Helicoverpa armigera – a characteristic pest in Hungary – has not been assessed in the dossier. We believe that there is a need on further information in this regard.	See section 6.1.3 (interaction of the GM plant with target organisms) of the scientific opinion. "*In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein expressed in plants. Therefore, the EFSA GMO Panel recommends these species are considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires*".
Hungary	Ministry of Environment and Water	D, 10.05 Interactions of the GM plant with non-target organisms	II. Furthermore, the risk assessment of the documentation regarding non-target organisms takes as its starting-point that among beneficial parasitoids and predators there are no species which can be adversely affected by MON 810. In contrast, assessment of more scientific studies has given evidence that Hymenopteras (parasitoids belong to this group) populations can significantly be decreased (Marvier, M., McCreedy, C., Regetz, J. & Kareiva, P. (2007): A meta-analysis of effects of Bt cotton and maize on non target invertebrates. Science, 316: 1475-1477.). The documentation does not contains detailed information on the potential adverse effect of MON 810 on Apis mellifera which collects MON 810 pollen containing Cry1 toxin and uses it for covering the protein needs of the brood. We believe that there is a need on further and detailed information in this respect. The documentation states that MON810 poses negligible risk to the environment. Contrarily, we would like to draw the attention to those data which show that the remains of maize may be transported to streams with wind and water where it may cause adverse effects. This is important because Trichoptera species, who were subject of the above mentioned	Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion. See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902002009.htm).

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			research, are the nearest relatives of butterflies. There are published data which shows that the Cry1Ab gene is detectable up to 21 days from live water and up to 40 days from sediment (Douville M., Gagné F., Blaise C., André C. (2007): Occurence and persistence of Bacillus thuringiensis (Bt) and transgenic Bt corn cry1Ab gene from an aquatic environment. Ecotoxicology and Environmental Safety 66: 195-203). According to the results of laboratory tests, the consumption of Bt maize producing the Cry1Ab toxin can cause negative effects on Trichoptera species that are frequent in streams. There were two species that was affected by Bt maize in growth rate or mortality. (Rosi-Marshall E.J., Tank J.L., Royer T.V., Whiles M.R., Evans-White M., Chambers C., Griffiths N.A. (2007): Toxins in transgenic crop byproducts may affect headwater stream ecosystem. Proceedings of the National Academy of Sciences USA 104: 16204-16208).	
Hungary	Ministry of Environment and Water	D, 10.05 Interactions of the GM plant with non-target organisms	I. (Technical dossier, Part I, D 9. 5. - Effects on non-target organisms) The Monsanto documentation simply mixes results of different studies as if all would state the same. (E.g.: the text contains: "However, field studies conducted over the past decade by industry and the academic community and reported in the peer-reviewed literature on registered insect-protected crops that produce a variety of Cry1A proteins, including Cry1Ab, have demonstrated that these crops have no adverse effects on biodiversity, tested populations of natural enemies, and other ecologically important non-target arthropods (U.S. and other world areas: (Daly and Buntin, 2005; Dively, 2005; Dively and Rose, 2003; Head et al., 2001; Head et al., 2005; Lozzia et al., 1998; Naranjo et al., 2005; Naranjo, 2005a; Naranjo, 2005b; Orr and Landis, 1997; Pilcher et al., 1997;	Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion. See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902002009.htm).

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Pilcher et al., 2005; Torres and Ruberson, 2005; Whitehouse et al., 2005) (E.U.: (Arpas et al., 2005; Babendreier et al., 2004; Bakonyi et al., 2006; Bourguet et al., 2002; Eckert et al., 2006; Freier et al., 2004; Heckmann et al., 2006; Lang et al., 2004; Ludy and Lang, 2006a; Ludy and Lang, 2006b; Meissle et al., 2005; Romeis et al., 2004; Romeis et al., 2006; Toth et al., 2004; Vercesi et al., 2006; Vojtech et al., 2005; Volkmar and Freier, 2003; Wandeler et al., 2002)). There is a reference to a Hungarian peer-reviewed article (Bakonyi et al. 2006) as it would underline that the genetically modified MON810 maize line do not have any effects on non-targeted species. On the contrary, our article call the attention that (a) potential negative effects must be evaluated on species-level because different species react differently, (b) during the feeding of a certain Collembola species, it gave a preference to the isogenic line instead of the Bt-maize. We can notice the same problem with the above mentioned article of Meissle et al. 2005: they also have found statistical significant differences. The study of Head et al. (2005) has been carried out on cotton, not on maize. We can not accept these results in regard of the maize authorization documentation. Scientific articles are quoted selectively in the Technical dossier. The text states that no adverse effect of Cry1Ab protein has been identified on soil organisms, such as Collembola, Lumbricidae and Nematoda species) (see page 133.) There are six quoted documents. The first document does not deal with this subject at all (Blackwood and Buyer 2004), the second one gives secondary attention to the issue (Motavalli et al. 2004), the third one is a report, not a peer-reviewed article (Evans 2002). There are only three cited documents dealing with soil animals, meanwhile there is no reference to other	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			significant articles of the particular issue.	
Hungary	Ministry of Environment and Water	D, 10.05 Interactions of the GM plant with non-target organisms	III. Furthermore, the Cry1 toxin containing pollen can get to the surface of leaves of other plant species living in the neighbourhood of the maize field. Protected insects living/feeding on these plants can also be affected (see Darvas B., Csóti A., Gharib, A., Peregovits L., Ronkay L., Lauber, É. and Polgár A. L. (2004): Adatok a Bt-kukoricapollen és védett lepkefajok lárváinak magyarországi rizikóanalíziséhez (Data for risk assessment of the Bt pollen on protected Lepidopteran larvae in Hungary), Növényvédelem, 40 441-449.). According to the Hungarian legislation on nature conservation, habitats of protected species such as Inachis io and Vanessa atalanta should be preserved. No changes in such habitats are tolerated by legislation. Habitats of both of the above mentioned Lepidopteran species can be affected and altered by MON 810 pollen as presented in the documentation supporting the Hungarian ban on MON 810. The Hungarian case differs from the "Danaus plexippus case" (Losey, J. E., Rayor, L. S. & Carter, M. E. (1999) Transgenic pollen harms monarch larvae. Nature, 399: 214.) – taking into consideration that Danaus plexippus is not protected in the USA. However, the proved negative effect on Danaus plexippus confirms the Hungarian findings that habitats of protected	Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion. See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902002009.htm).

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			Hungarian Lepidopteran species living on Urtica species can be negatively affected and significant reduction of the protected species can be monitored (Lang, A., Lauber, É. and Darvas, B. (2007): Early-tier tests insufficient for GMO risk assessment. Nature Biotechnology, 25: 35-36.). Data resulting from other Hungarian experiments show that MON 810 plant residues will not been degraded (see DT50) so fast as stated by Monsanto on page 134 (see: Székács, A., Juracsek, J., Polgár, L. A. and Darvas, B. (2005): Levels of expressed Cry1Ab toxin in genetically modified corn DK-440-BTY (YIELDGARD) and stubble. FEBS Journal, 272 Suppl. 1: 508.). This information shall also be incorporated into the risk assessment of MON 810. The chapter has the following conclusion: "In conclusion, there is negligible risk for harmful effects on MON 810 on non-target organism (vertebrates and invertebrates), either through direct or indirect interactions with this maize or through contact with the newly expressed protein Cry1Ab." We believe that there is a need on further information in this regard and request an itemized verification of the statement about the effects on Nematode from the notifier (cf. Höss, S., Arndt, M., Baumgarte, S., Tebbe, C.C., Nguyen, H.T., Jehle, J.A. 2008. Effects of transgenic corn and Cry1Ab protein on the nematode, Caenorhabditis elegans. Ecotoxicology and Environmental Safety 70: 334-340.).	

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Hungary	Ministry of Environment and Water	D, 10.08 Effects on biogeochemical processes	(Technical dossier, Part I, D 9. 8. - Effects on biogeochemical processes): The chapter only quotes two references. However, these are not of real relevance in terms of the subject. In the meanwhile there are no scientific publication referred in the documentation in order to underline the statements of this chapter ("Estimation of the risk it is highly unlikely that there is any difference between MON 810 and conventional maize with respect to its direct influence on soil nutrient levels and key processes"). The text does not contain any data on the Cry1Ab toxin in spite of the fact that there are several significant publications about the effects of MON810 varieties on soil processes (CO2 production, decomposition, mineralization, etc.) (Flores, S., Saxena, D., Stotzky, G., 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37: 1073–1082.; Castaldini, M., Turrini, A., Sbrana, C., Benedetti, A., Marchionni, M., Mocali, S., Fabiani, A., Landi, S., Santomassimo, F., Pietrangeli, B., Nuti, M.P., Miclaus, N., Giovannetti, M., 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. Applied and Environmental Microbiology 71: 6719–6729. etc.). There is a need on further information an in depth analysis of all available scientific data on the decomposition of MON810.	See section 6.1.6 (potential interaction with the abiotic environment and potential effects on biogeochemical processes) of the scientific opinion. See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902002009.htm).

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Hungary	Ministry of Environment and Water	D, 11 Potential interactions with the abiotic environment	(Technical Dossier, Part I. D 10. - Potential interactions with the abiotic environment): According to the text, the Cry1Ab toxin will rapidly been degradated in the soil. We strongly disagree with this statement. This toxin will rapidly be absorbed on the surface of minerals and humus materials which renders the protein resistant to biodegradation and remains active for long time (Stotzky G. (2004): Persistence and biological activity in soil of the insecticidal proteins from Bacillus thuringiensis, especially from transgenic crops. Plant Soil 266:77-89.). The toxin was detectable from the remains of maize for three years after the harvesting Flores, S., Saxena, D., Stotzky, G., 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. Soil Biology & Biochemistry 37: 1073–1082). It is of utmost importance to make an up-to-date review of new scientific results in the documentation as well as to assess these identified effects (see Icoz, I., Stotzky, G. 2008. Fate and effects of insect-resistant Bt crops in soil ecosystems. Soil Biology and Biochemistry 40: 559-586.).	See section 6.1.6.1 (persistence of Bt-proteins in soil) of the scientific opinion. See also the scientific opinion of the GMO Panel on the Hungarian safeguard clause on maize MON810.
Hungary	Ministry of Environment and Water	D, 12 Environmental Monitoring Plan	D 11.4.2: Protection goals in the framework of general surveillance includes ecological system and biodiversity as well as soil function. How will be able a farmer recognize about 800 overground animal species (Meszaros, Z. (Editor), (1984) Results of faunistical studies in Hungarian maize stands. Maize Ecosyst. Res. No. 16. Acta Phytopathol. Acad. Sci. Hung., 19: 65-90.) and about 200 underground animal species that exist in maize field? Will farmers get some information on how to monitor the parameters of soil functions (such as soil respiration, decomposition, etc.), or this work will be carried out by experts?	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				in each country should not be included in the original application. *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Hungary	Ministry of Environment and Water	D, 12 Environmental Monitoring Plan	(Technical Dossier, Part I. D 11. - Environmental Monitoring Plan): It seems that the monitoring of the environmental effects are planned to be fulfilled by farmer questionnaires. We strongly believe that this method is not appropriate for this purpose, because farmers are not qualified in environmental assessment.	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on Post Market Environmental Monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State.*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				(…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…). See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Ireland	Environmental Protection Agency	General comments	The Irish Competent Authority (CA) under Directive 2001/18/EC wishes to make the following comments in relation to the ERA part of this application (Ref- EFSA-GMO-RX-MON810): 1. The Irish CA is of the view that the applicant should provide a case specific post market monitoring plan (CSM) that should be implemented in the EU MS where this GMO is likely to be cultivated to meet the following objectives: v Confirm that any assumptions regarding the occurrence and impact of potential adverse effects of the GMO or its use in the environmental risk assessment are correct; and v Identify the occurrence of adverse effects of the GMO or its use on human health or the environment, which were not anticipated in the environmental risk assessment (ERA). We have noted that Germany suspended its ban on MON810 based on the agreement that the applicant provides extra monitoring data for the national competent authority. We suggest that the details of this extra case specific monitoring (CSM) be circulated to all Member States Competent Authorities for their comments. 2. The applicant be advised that the concept of "substantial equivalence" is not advocated under Regulation 1829/2003 and that all new GMO's must under go a case-by-case authorisation process. 3. The Irish CA requests clarification regarding the following aspect pertaining to Part	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC".* Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. 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*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			II -Summary of the Application: · Given that this is a renewal application it has been noted that there is a minimal amount of analysis or summary of findings since the original application. Details of any adverse effects noted since 1998, through general surveillance, should be submitted. · Section 7.1 & 7.2 – clarification is required regarding the growing dates for the comparative assessment – section 7.1 indicates 2004 & 2005 while section 7.2 makes reference to 1994 & 1995. · Further detail should be provided on * The statement "…the dietary safety of Cry1AB protein confirmed by animal feeding studies in the rate and broiler chickens" in Section 7.8.4. * The "harmonised Insect Resistance Management (IRM) stewardship programme" in Sections 9.4 & 9.9. · References or qualification are required for * The "numerous studies, which establish that Cry1AB exhibits toxicity to specific Lepidoptera" referred to Section 9.5; * The statement "the Cry1AB protein is subject to rapid degradation in the soil", again when one considers that other studies have indicated a certain level of persistence in the soil (Zwahlen et al 2003). Interaction with micro-organisms is also not really addressed – in Section 9.8; * The statement "no known negative effects on biochemical processes" in Section 10.	*by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant. Additional information has been requested to the applicant by the Spanish Competent Authority and by the GMO Panel on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion. See section 6.1.6.1 (persistence of Bt-proteins in soil) of the scientific opinion. See section 6.1.6 (potential interaction with the abiotic environment and potential effects on biogeochemical processes) of the scientific opinion.
Italy	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	As explained in earlier comments on notifications regarding the event MON810 (EFSA-GMO-UK-2004-01) there is discordant data in literature on its molecular characterization, therefore we require further clarification concerning the characterization of the event and its stability genomics.	Updated analysis of ORFs indicated no hypothetical chimeric proteins and no homologies with potential toxins or allergens, confirming the original bioinformatic assessment. However, the updated bioinformatic analyses did reveal that one ORF, previously identified as sharing homology with the importin protein, shared a higher level of identity to a more recently sequenced protein, the HECT-ubiquitin protein. There is phenotypic and compositional equivalence between MON 810 maize

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
				and its conventional counterparts so there is no evidence of any safety implications resulting from the interruption of this gene sequence.
Italy	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	D. Information relating to the GM plant	The MON810 maize produces a factor which provides for resistance to antibiotics. As it is known, Dir. 2001/18/EC has provided for commercial releases phasing out of products that contain these factors by the end of 2004. Although some factors may provide resistance to antibiotics which have no therapeutic value, the antibiotics to which the notifier affirms protein NPTII provides resistance, kanamycin and neomycin, are both included in the medical and/or veterinarian list of authorised pharmaceutical products. Since it is obvious that these lists contain only pharmaceutical products useful for therapeutic purposes, it does not appears appropriate to authorise GM products which contain resistance factors for such antibiotics. For this reason, this National Competent Authority believes that it is not possible to grant the authorisation requested.	There is no *npt*II in MON810.
Norway	Directorate for nature management	General comments	According to the Norwegian Gene Technology Act possible contributions to sustainable development and possible benefits to the society and ethical considerations through the use of a GMO, shall be taken into consideration when evaluating a GMO notification in Norway. Thus, we would, in order to facilitate an approval in Norway, like the applicant to elaborate on the effects of MON 810 on these subjects	(-)
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	In 11.4.2.4 the applicant states that general surveillance at regional and/or national levels is considered to be a national/European responsibility. We would like the applicant to provide the legal basis for this consideration.	(-)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	The internet address regarding details of the stewardship commitment of the authorisation holder given at the bottom of page 162 of the dossier seems to be invalid. Please correct.	(-)
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	The applicant claims that 2500 questionnaires, including a 10-20% drop out quota, spread over the monitoring period of 10 years are sufficient to provide statistical power to detect adverse effects of MON 810. Is the drop out quota of 10 – 20% based on empirical data? What actions are taken by the applicant to minimize the number of missing questionnaires? Will the applicant commit to raising the number of questionnaires the following years if the drop out quota exceeds 20%?	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). The GMO Panel comments on the scientific quality of the monitoring plan. See section 5.2 of the PMEM opinion (EFSA, 2006b): *Details of the specific plans and methods of monitoring in each country should not be included in the original application.* *The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…).* See section 6.2 of the scientific opinion and the recommendations proposed by the GMO Panel to improve the PMEM proposed by the applicant.
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	We agree with the applicant that the monitoring activities should reflect the level of market penetration of MON 810. On the other hand it is of great importance that the monitoring activities cover the full scale of ecosystems where cultivation of MON 810 takes place, and especially ecosystems containing endangered non-target species.	(-)
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM	On page 159 of the dossier it is stated that an example of a questionnaire for MON 810 is presented in Appendix 2. We are not able to	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
		plant	locate the Appendix or a questionnaire in the dossier. However, a questionnaire for the 2006 season was found in Annex 5 of the "Monitoring report, MON 810 cultivation. Czech Republic, France, Germany, Portugal and Spain 2005" supplied in Annex 2b in the folder specific information of this application. As the questionnaires seem to be improved on the basis of experience from previous years, we ask the applicant to provide the questionnaires intended for use in 2008.	(PMEM). An updated questionnaire (2007) for general surveillance was provided.
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	The applicant states that the time-period for general surveillance should be in line with the period of consent, i.e. maximum 10 years. Council decision 2002/811/EC states that the applicant should consider whether it is necessary to extend the monitoring plan beyond the period of consent. Such an extended period of monitoring could prove essential to detect delayed and/or indirect effects of the release of MON 810. We would like the applicant to comment on which considerations are done regarding the time-period for general surveillance, leading to the limitation of surveillance to the period of authorisation.	According the EFSA guidance document (2006), the GMO Panel recommend the applicant to submit " *i) annually confirming that monitoring has been carried out according to the given consent together with a major preliminary results that are important for a short-term feedback on ERA; ii) periodically (every third years) covering longer periods in which observations and data collected are reported and analysed in details and which therefore provide more comprehensive reports that are important for a longer term feedback on the ERA*".
Norway	Directorate for nature management	D, 12.03 General Surveillance of the impact of the GM plant	According to the 2006 MON 810 monitoring report, 1 of 4 Spanish farmers planting more than 5 ha MON 810 reported that they have failed to plant refuges, which they through the IRM plan were obliged to. The high level of non compliance to the IRM plan occurs despite efforts through several years by the applicant to emphasise the need for refuges to the Spanish farmers. The applicant states that the non compliance seen in Spain might relate to the Spanish history of Bt maize introduction. If there is a national scepticism towards the implementation of the IRM plan, questions	See section 6.2.3 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC*". *"The EFSA GMO Panel advises that the evolution of resistance in lepidopteran target pests continues to be monitored in order to detect potential changes in resistance levels in pest populations. In areas where other lepidopteran pests are important targets of maize MON810, they might also be subject to resistance evolution due to exposure to the Cry1Ab protein*

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			regarding the reliability of the farm questionnaires indicating that refuges have been planted could be raised. Are any actions taken by the applicant to ensure that the answers given through the farm questionnaires are correct?	*expressed in plants. Therefore, the EFSA GMO Panel also recommends these species to be considered by the applicant in the context of both case-specific monitoring for insect resistance management strategy and general surveillance through farm questionnaires".*
Slovenia	Ministry of Agriculture, Food and Forestry	General comments	1. We consider that applicant should submit data on long term effects and effects on subsequent generations to fulfill legal requirements on safety from Regulation (EC) No. 1829/2003 Article 4 (1 and 3), Regulation EC No 178/2002 and Annex II of Directive 2001/18/EC. 2. We would like to ask for clarification of the actual situation regarding information we got that MON 810 contains the NPTII gene that confers resistance towards the antibiotics: kanamycin, neomycin, geneticin, gentamicin A & B, butirosin and paramomycin, because this is not included in the dossier. In Slovenia there is a certain use of these antibiotics in human and veterinary medicine. In our opinion antibiotic resistance genes, including NPTII, should be phased out in accordance with article 4 (2) of directive 2001/18/EC. 3. Statistically significant differences from 90 days subchronic toxicity study must be further addressed to exclude potential negative health effects.	Additional information has been requested to the applicant by the molecular characterisation group, food-feed group and environmental group. Long-term effects are addressed in several sections of the opinion. General surveillance programs will identify possible unexpected adverse effects during commercialization
Sweden	The Swedish Board of Agriculture	D, 03 Information on the expression of the insert	In view of the discussions about non-target organism effects due to dissemination of pollen, data on concentrations of Cry1A(b) in pollen should be presented by the notifier.	(-)
The Netherlands	Ministry of Housing, Spatial Planning and the	D, 03 Information on the expression of the insert	The data provided in the dossier on phenotypic and agronomic traits and on expression levels of the Cry1Ab protein are derived from field trials conducted in 1994 and 1995. Although the provided monitoring reports from recent years	The EFSA GMO Panel has already assessed the agronomic and phenotypic characteristics of maize MON810 in relation to an appropriate non-GM maize control having a comparable genetic background in connection with giving its opinions on several stacked

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
	Environment		confirm the safe use of maize event MON 810 it would be useful to update the data set.	events (EFSA, 2005a,b,c,d,e). The information available in the present renewal application gives no reason to change the opinion that maize MON810 is agronomically and phenotypically equivalent to currently grown non-GM maize varieties, with exception of the insect resistance conferred by the Cry1Ab protein. The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of the plant.
The Netherlands	Ministry of Housing, Spatial Planning and the Environment	D, 07.04 Agronomic traits	The data provided in the dossier on phenotypic and agronomic traits and on expression levels of the Cry1Ab protein are derived from field trials conducted in 1994 and 1995. Although the provided monitoring reports from recent years confirm the safe use of maize event MON 810 it would be useful to update the data set.	The GMO Panel has considered all available information submitted by the applicant as well as scientific publication and monitoring reports on MON810. Specific results about agronomic performances are available in the frame of variety registration processes (about 90 varieties expressing this trait are already available). The GMO Panel considered this set of information sufficient for the classification of the agronomic characteristics of the plant.
United Kingdom		D, 10 Potential changes in the interactions of the GM plant with the biotic	Trophic effects: Hillbeck et al 1998 (Env. Entomol, 27, p.1255) showed a significant effect, not connected to prey quality, on survival rates of Chrysoperla carnea reared on 100 mg/ml of Cry1Ab toxin. In itself the observation is of minor interest, since the number of prey larvae that have fed on Bt-plants should be very low in Bt-maize fields as a consequence of that the levels of Bt-toxin in the plants is lethal. However, the observation could be of interest for predators on other herbivores on Bt maize, herbivores that are unaffected by the Bt-toxin without breaking it	Additional information has been requested to the applicant by the Spanish Competent Authority and by the environmental working group on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.

Country	Organisation	Reference	Comment	EFSA GMO Panel response
Comments from National Competent Authorities under Directive 2001/18/EC				
			down. As a result the predators may ingest large amounts of Bt-toxin. Since then other studies have not been able to detect an effect not connected to prey quality and also, C. carnea preying on the unsusceptible prey Tetranychus urtica containing large amounts of Bt-toxin were not effected (Dutton et al. 2002, Ecol. Entomol, 27, p.441). This was also the case for Orius majusculus feeding on the unsusceptible Anaphothrips obscurus in Zwahlen et al. 2000 (Env. Entomol, 29, p. 846). The Swedish Board of Agriculture would like EFSA:s GMO-panel to discuss the observations of Hillbeck et al. 1998, which deviate from the general pattern, and comment on if it constitutes an indication of risks for non-target predatory insects? Direct effects on non-target organisms from distributed plant materials: In the aplication no effect of was found on the lady beetle Hippodamia convergens when it was fed activated Cry1A(b) toxin. This is in contrast to Schmidt et al. 2004 (Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie, 14, p.419) where the lady beetle Adalia bipunctata showed increased mortality when feeding on eggs sprayed with activated Cry1A(b) toxin. In the same article the lady beetle Stethorus punctillum was unaffected by feeding on Tetranychus urticae which fed on Cry1A(b)-expressing maize. Is there a species difference in susceptibility within the lady beetles? Or are the differences due to different experimental protocols? We would like the GMO-panel to comment on this. The aplicant should present data on which insect species in the order lepidoptera that normaly visit or live in or close to maize fields in the EU. Monitoring of a species should be considered, selected on the basis of susceptibility and feeding pattern. Rosi-marshall et al. 2007 (PNAS 104, p. 16204) have produced	See section 6.1.4.5 (Effects on non-target water-dwelling organisms) of the scientific opinion.

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
Country	**Organisation**	**Reference**	**Comment**	**EFSA GMO Panel response**
Comments from National Competent Authorities under Directive 2001/18/EC				
			results that indicate possible effects on aquatic insects, such as caddis flies. Since this is a first experiment, some issues are pending. Nevertheless, a science based suspiscion is fulfilled according to the requirements of the precautionary principle. The question must therefore be resolved. Additional experiments are under way. We would like the GMO-panel to assess the biological relevance of the present findings as well as additional findings, discuss putative further experiments needed and to consider a meaningful monitoring action. Effects on soil: Zwahlen et al. 2003 (Mol.Ecol, 12, p.765) observed that tillage reduced the degradation of Bt-toxin (as detected by ELISA) within plant residues. This is no risk in itself. Three decomposers were extracted in higher numbers from non-Bt than Bt plant residues in another study, Zwahlen et al. 2007 (Plant and soil, 300, p.245). In the aplication, the worm Eisenia fetida is unaffected by Cry1A(b), while in Zwahlen et al. 2003 (Mol.Ecol, 12, p.1077) there is a slight effect on the worm Lumbricus terrestris. The putative risk is connected to whether some species of decomposers are sensitive to Bt-toxin and thus would be discriminated. The Swedish Board of Agriculture would like the GMO-panel to analyze this risk.	See also minutes of the 37th Plenary meeting of the GMO Panel where the publication of Rosie-Marshall (2007) was assessed (http://www.efsa.europa.eu/cs/BlobServer/Event_Meeting/GMO_Minutes_37th_plenmeet.pdf?ssbinary=true)

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
United Kingdom	Defra	D, 10.04 Interactions between the GM plant and target organisms	The applicant has indicated that the PMM plan will continue to be directed at farmers growing over 5 hectares of Bt maize. This approach should be reconsidered at intervals to take into account the amount and distribution of Bt maize (resistant to the same pest) that is cultivated and the extent of adoption of non-GM maize refugia by farmers.	The GMO Panel agree that representative farm size is used in the context of PMEM. See section 6.2 of the scientific opinion *"The EFSA GMO Panel recommends that resistance management strategies continue to be employed and case-specific monitoring is conducted by the applicant under Directive 2001/18/EC"*. The GMO Panel indicates that the reasons for implementing the *refugia* on farms where the Bt-maize area is greater than 5ha are: the high fragmentation of the European agricultural landscape; the lack of economic feasibility for providing *refugia* on farms with less than 5ha Bt-maize; and the negligible risk of resistance development in Bt-maize areas smaller than 5ha.
United Kingdom	Defra	D, 10.05 Interactions of the GM plant with non-	The UK considers that the evidence submitted on the potential for adverse effects on non-target organisms in this application could have been	Additional information has been requested to the applicant by the Spanish Competent Authority and by

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
		target organisms	better presented and discussed. A full risk assessment for non-target Lepidoptera likely to be found in and around maize fields in Europe has not been presented. Studies directly relevant to the EU should have been separated from those specific to other parts of the world. The concentration of Cry1Ab protein in MON810 maize pollen is not provided in the section on protein expression in the technical dossier.	the GMO Panel on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion.
United Kingdom	Defra	D, 12.03 General Surveillance of the impact of the GM plant	To note in section 11.4.3.3 on page 162, areas to be monitored refers to 'the glyphosate tolerance trait'.	(-)
United Kingdom	Defra	D, 12.04 Parameters to be used in a monitoring plan	The most important factor in general surveillance is detecting an adverse effect rapidly so that it can be reversed as quickly as possible. For questionnaires, coverage is the most important factor with questions kept direct and simple. However, in this case, given the insecticidal properties of MON810 maize, UK considers that the question on 'wildlife' is not specific enough. It should prompt farmers to look for changes in insect abundance in and around MON810 maize fields.	Additional information has been requested to the applicant by the Spanish Competent Authority in relation to the Post Market Environmental Monitoring plan (PMEM). An updated questionnaire (2007) for general surveillance was provided.
United Kingdom	Defra	D, 10.05 Interactions of the GM plant with non-target organisms	The UK considers that the evidence submitted on the potential for adverse effects on non-target organisms in this application could have been better presented and discussed. A full risk assessment for non-target Lepidoptera likely to be found in and around maize fields in Europe has not been presented. Studies directly relevant to the EU should have been separated from those specific to other parts of the world. The concentration of Cry1Ab protein in MON810 maize pollen is not provided in the section on protein expression in the technical dossier.	Additional information has been requested to the applicant by the Spanish Competent Authority and by the GMO Panel on the interaction between the GM plant and TOs and NTOs. See sections 6.1.3 (interaction of the GM plant with target organisms) and 6.1.4 (interaction between the GM plant with non target organisms) of the scientific opinion. Exposure assessment for some non target Lepidoptera species has been conducted by the GMO Panel based on a simulation model (see paragraph 6.1.4.2. (b))

Application EFSA-GMO-RX-MON810 (20.1a cultivation) ANNEX G Comments and opinions submitted by Member States during the three-month consultation period				
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Comments from National Competent Authorities under Directive 2001/18/EC				
United Kingdom	Defra	D, 12.06 Reporting the results of monitoring	The questionnaires should be analysed and the results made available to regulators as soon as is practical and preferably before the next crop is sown.	In line with the EFSA guidance document, the applicant is requested to report annually and periodically (every 3d year).