Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	General comments	Federal Ministry of Health	The whole statement (i.e. all comments submitted through the EFSA GMO Extranet) refers also to confidential business information!	

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	General comments	Federal Ministry of Health	In this notification the applicant presents studies regarding phenotypic and agronomic characteristics (Technical Dossier, p. 105 ff.) and ecological interactions (Technical Dossier, p. 110 ff.) in particular to establish physiological data and assess specifically the potential for weediness, dissemination and survivability of GM maize MON87460. Concerning e.g. the field trials conducted for the phenotypic and agronomic characteristics but also for the comparative assessment, information and data concerning one particular study were presented separately in different reports (e.g. one report on the management of the trial and another report to evaluate the established data). Therefore, relevant information on a single issue needs to be collected from various individual reports, if available in the submitted documents (see comment on agronomic, expression and compositional assessment). We request that the notifier does pay attention to the way the information is presented in the Technical Dossier (and the relevant study reports). The Technical Dossier – or any Annex summarising a particular assessment - should clearly indicate what was assessed where, when and how. We, therefore, request that the notifier clearly presents this basic information for each information element required by the EFSA Guidance Document (EFSA 2006) in the Technical Dossier. In this respect, we also recommend that the Technical Dossier should contain a table providing an overview of the studies conducted for a specific assessment indicating which data have been established in a certain trial. In this notification, an example for such a table can be found on page 122 (Tab. 15) giving an overview of the field sites used for the phenotypic evaluation. However, references to the respective studies are missing. Such tables should reference the report which contains the respective data in order to facilitate evaluation.	1/ EFSA refers to Appendix D of its guidance document on the submission of GM plant market approval applications submitted under Regulation (EC) No 1829/2003 (http://www.efsa.europa.eu/en/efsajournal/doc/2311.pdf), and reinforces the requirement to provide a schematic summary for each field/greenhouse trial conducted for the comparative analysis of agronomic and phenotypic characteristics in forthcoming applications. 2/ EFSA refers to Table 2 of the EFSA GMO Panel Scientific Opinion, as it provides an overview Table of the compositional field trials, agronomic and phenotypic field trials, abiotic stress response studies, persistence and invasiveness assessments, seed germination tests, and pollen morphology and viability tests provided by the applicant.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	General comments	Federal Ministry of Health	The Technical Dossier suffers in addition from the following shortcomings: 1) Because of a lack of useful data a final decision on the long-term safety of the newly expressed proteins (CspB, NptII) cannot be made. 2) Concerning comparative assessment important information on the management of the sites, sampling procedures and weather conditions is missing. 3) The decision of the notifier to avoid the removal of the antibiotic resistance marker gene from the adult plant genome is disconcerting. Knowing about the strict opposition of some stakeholders concerning the use of ARM genes and having the possibility at hand to avoid these troubles by simply applying the already implanted technology the notifier's approach is scientifically not state of the art. By acting in this way the notifier shows no interest in removing a potential risk factor and in a facilitation of the overall risk assessment process. It is incomprehensible why the notifier had taken the burden of the development of a complex transformation vector containing an nptII expression cassette flanked by two loxP sites, which allow the excision of the DNA in between (and, thus, the removal of the nptII antibiotic resistance marker gene) and then decided to neglect this implemented technology. The decision of the notifier to proceed without applying the present technology is irresponsible and, thus, inacceptable. 4) The use of nptII as antibiotic resistance marker gene is highly contestable on scientific grounds and can as well not be regarded as the latest state of the art, as its removal is possible. It further stands in contrast to the speech of Commissioner Dalli on 30 March 2010 in the Crop Life Conference where he demanded a complete phase out of antibiotic resistant marker genes. [EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100.]	Having considered the information provided in the technical dossier and the additional information provided by the applicant on 30/04/2012 upon request of the EFSA GMO Panel, as well as relevant publications published in the scientific literature, the EFSA GMO Panel concluded that adverse effects on human and animal health and the environment resulting from the transfer of the *npt*II and *csp*B genes present in maize MON 87460 to bacteria are unlikely, because of a highly limited potential for gene transfer. Taking into account the different exposure routes, this conclusion is mainly based on the following assessment: (1) the integration of the *npt*II and *csp*B genes through non-homologous recombination is most unlikely; (2) enhanced horizontal transfer of the *npt*II gene due to Cre-*lox* mediated recombination is unlikely; (3) the stabilisation of the *npt*II gene into bacterial cells by double homologous recombination of *A. tumefaciens* sequences flanking the *npt*II gene, and subsequent dissemination in the environment are unlikely; and (4) the unlikely but theoretically possible transfer of the *npt*II and *csp*B genes in maize MON 87460 to bacteria via gene replacement does not raise concerns due to the lack of an additional selective advantage which would be provided to the recipients in the receiving environments. The probability of horizontal gene transfer of the insert DNA of maize MON 87460 remains several orders of magnitude lower than the gene transfer efficiencies between bacteria. Therefore, its contribution (if any) to the environmental prevalence of *npt*II genes is negligible. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
				a risk to human or animal health or to the environment in the context of its intended uses.
Austria	General comments	Federal Ministry of Health	Detection Method: The provided detection method describes an event specific method for detection of GM maize MON87460, i.e. the application of the proposed PCR setup allows amplifying a fragment corresponding to the transgene insert and the maize genome. The used primers and probes are specific for GM maize MON87460. Specificity was demonstrated empirically by tests with different Monsanto products. This method is currently under validation by the EURL-GMFF (http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm). The validation process has not yet been completed and is currently on step 2 "(scientific assessment) completed". Additionally, Monsanto carried out an in-house validation that is presented in the notification papers. This validation report meets the required specifications. Notwithstanding, before placing on the market of this product, a validated detection method should be published.	Not in the remit of the EFSA GMO Panel.
Austria	C, 01 Description of the methods used for the genetic modification	Federal Ministry of Health	Table 2, p. 33: Please replace "3'(9)-O- nucleotidyltransferase" by the correct designation "3''(9)-O- nucleotidyltransferase" (Fling et al. 1985; Mingeot-Leclercq et al. 1999). [Fling, M. E., Kopf, J. and Richards, C. (1985). Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-O-nucleotidyltransferase. Nucleic Acids Res 13(19): 7095-7106. Mingeot-Leclercq, M. P., Glupczynski, Y. and Tulkens, P. M. (1999). Aminoglycosides: Activity and resistance. Antimicrob Agents Chemother 43(4): 727-737.]	The GMO Panel acknowledges the clarification. The designation of the gene will not affect the outcome of the risk assessment.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	C, 03 Size, source (name) of donor organism(s) and intended function of each	Federal Ministry of Health	Page 35: The notifier maintains that "… there is no evidence of any safety issues related to the use of the genetic elements…". The utilisation of the nptII expression cassette is unnecessary, has no benefit for the adult plant and bears the risk for replenishing the resistance gene pool with this aminoglycoside resistance determinant in receptor bacteria interfering with effective antimicrobial chemotherapy during treatment of animal and human infectious diseases. The expression "…no evidence of any safety issues…" is misleading and should be replaced by discussing the problems inherent to horizontal gene transfer of ARM genes. The notifier maintains that "DNA … is quickly degraded by restriction nucleases present in the gastrointestinal tract of humans and animals to nucleic acids." The intention of the notifier expressing this sentence is unclear. DNA (= deoxyribonucleic acid) is a nucleic acid. Degradation to "nucleic acids" makes no sense. Moreover, there is a huge amount of literature available providing evidence that orally administered DNA is not completely degraded in gastrointestinal fluids for a certain period of time and survives - albeit reduced in length - the passage through the gastrointestinal tract (Schubbert et al. 1994; Schubbert et al. 1997; Schubbert et al. 1998; Martin-Orue et al. 2002; Netherwood et al. 2004; Wilcks et al. 2004; Sharma et al. 2006; Alexander et al. 2007). The notifier maintains that "…there is no evidence that DNA from dietary sources has ever been incorporated into the mammalian genome." This statement is wrong, misleading and has to be replaced by citing several research papers providing evidence for DNA incorporation into mammalian genomes (Schubbert et al. 1994; Schubbert et al. 1997; Mazza et al. 2005; Alexander et al. 2007). [Alexander, T. W., Reuter, T., Aulrich, K., Sharma, R., Okine, E. K.,	The EFSA GMO Panel requested further information concerning the presence of the *npt*II gene and the possibility for horizontal gene transfer. Please see the EFSA GMO Panel Scientific Opinion and additional information provided on 04/10/2010 and 30/04/2012 for further details. *The scope of this application is for food and feed uses, import and processing, and excludes cultivation in the EU. Therefore, the route of DNA exposure is through consumption of maize MON 87460 material. Furthermore, exposure may occur via accidental spillage into the environment of maize MON 78460 grains during transport and processing. From all maize commodities imported in the EU, the whole maize grains and the maize flour are the most conceivable sources containing DNA of sufficient size to encompass full length gene sequences. In the other maize commodities, as maize gluten feed and meal, dregs from brewing and distilling and maize oil, the plant DNA is not detectable or intensively degraded to fragments with estimated lengths < 1500 bp (Rausch and Belyea, 2006; Rizzi et al., 2012). Therefore, the possible source of full length genes from maize MON 87460 to bacteria would mainly be limited to unprocessed whole grain partially digested or spilled during transit, and to maize flour.* *DNA present in food and feed becomes substantially further degraded through digestion in the human or animal gastrointestinal tract by host and microbial factors, and the likelihood that a full length gene sequence persists is very low in the lower intestinal tract (see references in Rizzi et al., 2008; EFSA, 2009).*

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			Dixon, W. T. and McAllister, T. A. (2007). A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production. Anim Feed Sci Technol 133(1-2): 31-62. Martin-Orue, S. M., O'Donnell, A. G., Arino, J., Netherwood, T., Gilbert, H. J. and Mathers, J. C. (2002). Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. Br J Nutr 87(6): 533-542. Mazza, R., Soave, M., Morlacchini, M., Piva, G. and Marocco, A. (2005). Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Res 14(5): 775-784. Netherwood, T., Martin-Orue, S. M., O'Donnell, A. G., Gockling, S., Graham, J., Mathers, J. C. and Gilbert, H. J. (2004). Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. Nat Biotechnol 22(2): 204-209. Schubbert, R., Hohlweg, U., Renz, D. and Doerfler, W. (1998). On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol Gen Genet 259(6): 569-576. Schubbert, R., Lettmann, C. and Doerfler, W. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. Mol Gen Genet 242(5): 495-504. Schubbert, R., Renz, D., Schmitz, B. and Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. PNAS 94(3): 961-966. Sharma, R., Damgaard, D., Alexander, T. W., Dugan, M. E. R., Aalhus, J. L., Stanford, K. and McAllister, T. A. (2006). Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem	EFSA, 2009. Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants. The EFSA Journal, 7, 1108 Rausch KD and Belyea RL, 2006. The future of coproducts from corn processing. Applied Biochemistry and Biotechnology, 128, 47-86. Rizzi A, Raddadi N, Sorlini C, Nordgard L, Nielsen KM and Daffonchio D, 2012. The Stability and Degradation of Dietary DNA in the Gastrointestinal Tract of Mammals: Implications for Horizontal Gene Transfer and the Biosafety of GMOs. Critical Reviews in Food Science and Nutrition, 52, 142-161.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			54(5): 1699-1709. Wilcks, A., van Hoek, A. H., Joosten, R. G., Jacobsen, B. B. and Aarts, H. J. (2004). Persistence of DNA studied in different ex vivo and in vivo rat models simulating the human gut situation. Food Chem Toxicol 42(3): 493-502.]	
Austria	C, 03 Size, source (name) of donor organism(s) and intended function of each	Federal Ministry of Health	1/ Page 36: It is not clear why a change in the triplet code for leucine (i.e. within the coding region) in the CspB protein leading to a change of the amino acid at position 2 should be necessary for a correct assembly of the transforming vector PV-ZMAP595. Please explain. 2/ LoxP sequences/sites: Why did the notifier not take advantage of the loxP sites flanking promoter and coding region for nptII to excise this antibiotic resistance marker gene? Obviously, removability of the flanked DNA sequence was the initial intention of the notifier. Please explain.	1/ Amino acid changes are common consequences of DNA cloning procedures and do not raise a safety concern *per se.* 2/ The EFSA GMO Panel requested further information concerning the presence of the *npt*II gene and the possibility for horizontal gene transfer. Please see the scientific opinion and additional information provided on 04/10/2010 and 30/04/2012 for further details.
Austria	D, 01 Description of the trait(s) and characteristics which have been introduced	Federal Ministry of Health	1/ Table 3, p. 42: The exponent in " LOD^2" is not explained. Please provide the missing information. 2/ Figure 6a, p. 43: The notifier maintains that "… the RNA was observed on 10% agarose gels…". Considering the nature (total RNA) and the length of the applied nucleic acid fragments and the physico-chemical characteristics of agarose, the use of a 10% agarose solution is rather unlikely. Please provide the correct agarose concentration. The molecular weight marker appears to be substantially degraded (see intense white spots in lane 1 at the bottom of the gel) and thus useless for the discrimination of fragments lengths. Please provide a gel photo which allows the discrimination of fragment lengths. At high CspB concentrations (50 µg; lane 7), the lower rRNA	1/ Please note that the abbreviation LOD is explained in the list of abbreviations: LOD Limit of detection. 2/ In materials and methods of Burzio (2008c) it is stated that a 1,2% gel is used.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			fragment seems to disappear. Please provide an explanation for this phenomenon	
Austria	D, 01 Description of the trait(s) and characteristics which have been introduced	Federal Ministry of Health	1/ Figure 6b, p. 43: A designation of the fragment lengths of the molecular weight marker is missing. Please provide the data. At high CspB concentrations (50 µg; lanes 4 + 8), the lower rRNA fragment seems to disappear. Please provide an explanation for this phenomenon. The notifier maintains that "no shifts are observed for increasing amounts of BSA." There was only one single concentration of BSA tested. Please provide a correct statement 2/ Table 4, p. 48 + 51: The notifier maintains that "… MON 87460 demonstrated improvements in yield and yield components through trends toward increased yield (16.5%), kernels per ear (13.1%), and kernel weight (3.9%) (Table 4)". Please indicate if these differences were significant or have been in the range of natural variation (considering the maize control and/or existing maize variants). 3/ The numeral data of some agronomic parameters presented in the main text do not correspond with the numbers displayed in the corresponding table 4 (e.g. yield: 16,5% (text) vs 16,4% (table); kernels per ear: 13,1% (text) vs 12,9% (table) and table 5 (e.g. yield: 9,3% (text) vs. 9,6% (table) etc…). Please provide the correct data. Page 53: The visual quality of the cited reference from "De Block et al., 1984" is inferior. It cannot be deciphered. Please provide a legible version of the paper.	1/ The aim of this assay is to analyse if the CspB can bind RNA and gel retardation was shown. This does not raise a safety concern. 2/ Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that under water-limited conditions, maize MON 87460 exhibited lower yields than in well-watered conditions but higher yields across locations compared with its conventional counterpart, though these differences were not consistently observed across studies and seasons. 3/ The figures in Table 4 correspond with those in the appendix Luethy (2009), which included a comparison of test and control, without inclusion of commercial reference varieties in the trial design. Besides this trial, many other trials focused on agronomic and phenotypic characteristics have been carried out and are summarized in section 4.1.3 of the EFSA GMO Panel Scientific Opinion. Luethy, M.H. (2009) Amended Report for MSL 0021720: Yield component and physiology data from 2003 Kansas and 2007 California field trials. Monsanto Technical Report MSL0022168. Monsanto Co. St. Louis, USA [as part of confidential dossier information]
Austria	D, 02 Information on the	Federal Minis	1/ The molecular characterization of GM maize MON87460 is based on a quite comprehensive analysis by Southern blot- and PCR-analyses as well as by sequencing of the insert and the genomic	1/ No endogenous homologous sequences were expected rendering the use of a near-isogenic control not essential. No unexpected hybridisation signals were observed and

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
	sequences actually inserted or deleted	try of Health	flanking sequences. The control line used for the molecular characterization experiments (Skipwith et al. 2007) is described without giving reference to the maize lines used for generation of control line GLP-0604-17133-S (sample number 65016471295) in the study itself. The Technical Dossier indicates on page 154 that line H1548126, which was derived by crossing parental strains LH244 and LH59, was used but does not indicate in which of the experiments the respective line was employed. Additionally, no reference is given whether this line is corresponding to the above mentioned control lines used by Skipwith et al. (2007). The notifier is therefore requested to clarify the identity of the control lines and to unambiguously reference them. 2/ Another issue of interest is whether the lox-elements present in GM maize MON87460 are still functional. The notifier should thus clearly indicate whether any differences between the sequences of the T-DNA present in plasmid PV-ZMAP595 and the transgenic sequences present in GM maize MON87460 were identified, specifically regarding any potential changes to the lox-elements. The information submitted by the notifier furthermore does not indicate how many different plants were sampled for the analyses, specifically for the analysis of genetic stability of GM maize MON87460 over several generations by Southern blot analysis as described in Skipwith et al. Furthermore, the analysis was performed in a way that only substantial changes to the inserted sequences could have been detected. It is requested that the notifier clarifies this issue and presents an analysis of the power of the submitted data with respect to estimation of the stability of the transgenic insert. [Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D.	therefore, the exact identity of the maize control line is not essential for the conclusion. 2/ The sequence of the lox-sites are conserved in maize MON 87460. "In order to assess the stability of the inserted DNA in maize MON 87460 across multiple generations, DNA isolated from different generations of seed was used." Southern analyses are considered sufficient to show genetic stability by the EFSA GMO Panel. In addition, the stability was also shown by segregation analyses using PCR based assays (Rosenbaum 2008). Rosenbaum, E.W. (2008) Assessment of insert segregation for MON 87460. Report number 07-RA-B3-01. Monsanto Co., St. Louis, USA [as part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			(2007). Molecular analysis of corn MON 87460. Dossier	
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	1/ Specific comments on study report MSL0020487 (Skipwith et al. 2007):Abbreviations and Definitions: Please use the correct designation for the aadA gene: 3''(9)-O-nucleotidyltransferase 2/ 3.2 Control Substance: A detailed description (e.g. breeding scheme) of the conventional maize control line which could provide evidence for the genetic relationship is missing. Please provide this information otherwise the suitability of the control substance cannot be evaluated. 3/ 3.4 Characterization of Test, Control and Reference Substances It is not clear why different quality criteria were applied for the assessment of the stability during storage of test and reference substance versus the control substance. The applied quality criteria were feeble considering the fact that amplicons from nearly completely degraded DNA samples may be obtained by PCR. 4/ 4.0 Results and Discussion: The migration rates of genomic DNA and molecular weight marker DNA were different. The explanation of the notifier for this aberrant behaviour ("different salt concentrations") is inacceptable, because the conclusions based on the Southern blots depend on the correct estimation of fragment sizes. Furthermore, the applicant should comment why the altered migration occurred only in "most of" - but not all of - the Southern blots (as stated on p. 19, first paragraph). The presented Southern blot photos should provide clear and simple evidence for the drawn conclusions and should not be the cause for additional uncertainties. Please provide Southern blots with correctly purified DNA templates.	1/ The EFSA GMO Panel acknowledged the clarification. The designation of the gene will not affect the outcome of the risk assessment. 2/ This information was provided by the applicant followed a request by the EFSA GMO Panel. 3/ No endogenous homologous sequences were expected rendering the use of a near-isogenic control not essential. No unexpected hybridisation signals were observed and therefore, the exact identity of the maize control line is not essential for the conclusion. 4/ The Southern analysis is used to determine the copy number and sequence analysis is used to determine detailed information on the insert junction sequence. For the Southern analysis different restriction enzyme probe combinations are used which together lead to the conclusions drawn by the applicant. Since the conclusions of the Southern analyses are confirmed by sequence analysis the EFSA GMO Panel did not regard that this raises a safety concern. In addition, the EFSA GMO Panel would like to refer to article of Southern (Southern E., 2006: Southern blotting. Nature Protocols 1(2):518-25) giving further insights into factors affecting the quality of Southern blots.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	Specific comments on study report MSL0020487 (Skipwith et al. 2007) (cont): 4.1.1 Southern Blot Analyses to Determine Insert and Copy Number: Figure 5: Please comment on the horizontal shift of the smear between molecular weight marker (MWM) indications 10 and 15, which are particularly obvious in lanes 10 and 11, less in lanes 8 and 9. Similarly, please comment on the colour deviations within the smear between MWM indications 8.1 and 10, lanes 3 and 4. Figure 5, lanes 4 + 11: The signal of the larger 7.2 kb fragment representing insert and 5' genomic border sequences is substantially weaker compared to the shorter 2.7 kb fragment. As the notifier maintains that there is only a single T-DNA insert, both fragments should show at least comparable intensities. Please explain the discrepancy. Figure 5, lanes 1, 3, and 8: These 3 lines show an undistinguishable smear. A serious interpretation of these lanes is impossible. [Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D. (2007). Molecular analysis of corn MON 87460. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	The applicant has provided Southern analyses showing absence of vector backbone sequences using separate probes (Song, 2010). The EFSA GMO Panel noticed that some imperfections can be observed in the Southern blots in Skipwith et al. (2007). However this is not abnormal as described by Southern (E Southern, 2006, Nature Protocols 1, 518-525). Taken together the results of the Southern analyses and the sequencing, the EFSA GMO Panel did not regard that this raises a safety concern. Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D. (2007). Molecular analysis of corn MON 87460. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Song, Z., et al. (2010) Confirmation of the absence of plasmid vector PV-ZMAP595 backbone sequence in the genome of MON 87460 by Southern Blot analysis. Report RAR-10-282. Monsanto Co., St. Louis, USA [as part of confidential dossier information, additional information received on 4 October 2010]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	Specific comments on study report MSL0020487 (Skipwith et al. 2007) (cont.): 4.1.2 Southern Blot Analysis to Determine the Presence or Absence of Plasmid PV-ZMAP595 Backbone Sequence Figure 5 and Figure 14: The sizes of DNA according to the MWM are not correct: Please compare Fig. 5, lanes 5 and 6 with Fig. 14, lanes 2 and 3; consequently, it is not correct that "the results were the expected bands at approximately 1.4, 1.6, and 2.0 kb", as stated on p. 21, second paragraph. Figure 6, lane 7: Please explain the substantial difference in the intensity of the bands. This discrepancy cannot be observed in lane 7 of Figure 5. 4.2.6 nptII Coding Sequence Probe: The notifier maintains that "…the migration of the approximately 5.5 kb fragment is slightly lower than indicated by the molecular weight marker band sizes" The expected size of the fragment is 6.1 kb the indicated difference in fragment length is approx. 10% and, thus, substantial. The notifier's explanation (different salt concentrations) is to be rejected. Please provide a conclusive identification of the 5.5 kb fragment.	The sizes are as expected. In addition, the applicant has provided Southern analyses showing absence of vector backbone sequences using separate probes (Song, 2010). Song, Z., et al. (2010) Confirmation of the absence of plasmid vector PV-ZMAP595 backbone sequence in the genome of MON 87460 by Southern Blot analysis. Report RAR-10-282. Monsanto Co., St. Louis, USA [as part of confidential dossier information, additional information received on 4 October 2010]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	1/ Specific comments on study report MSL0020487 (Skipwith et al. 2007) (cont.): 4.3.1 Southern Blot Analysis to Examine Generational Stability of the Insert: Figure 14: Lanes 1 and 2 show a strong background hybridisation in the high molecular weight range. There is rather no background visible in lane 3 concerning the same high molecular weight range. Please explain the discrepancy. Furthermore, please explain the additional signal at approx. 9 kb, lane 11. Figure 14, lane 3: Why is the intensity of the lowest band (approx. 1.4 kb) significantly lower compared to the higher molecular weight fragments? 2/ Lane 11: There are two additional high molecular weight fragments of approx. 9 and 10 kb (additionally to the expected 2 target bands at 2.7 and 7.2 kb), which are not visible in lanes 5-10. Indistinguishability may be due to the overall inferior quality of the blot photo, but at least the intensity of these two additional bands changed significantly in the last tested generation (lane 11). Please explain this unexpected result.	1/ The Southern blot displayed in Figure 14 has the purpose to show that the insert is stable. Although there is a quite strong background both expected bands can be clearly seen in all generations. Therefore, this blot can be used to show stability of the insert. 2/ The difference in intensity can result from differences in optimal annealing temperature or nucleotide composition between the different probes used. The most important fact to consider is if the fragment is clearly visible at 1 copy amount. As this is the case the EFSA GMO Panel did not consider this to raise a safety concern.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	1/ Specific comments on study report MSL0020487 (Skipwith et al. 2007) (cont.): 4.3.2 Southern Blot Analysis to Determine the Presence or Absence of Plasmid PV-ZMAP595 Backbone Sequence: Figure 6 and Figure 15: The sizes of DNA according to the MWM are not correct: Please compare Fig. 6, lanes 5 and 6 with Fig. 15, lanes 2 and 3; the comment given in the text (p. 28) is not sufficient. 2/ Figure 15: A strong background hybridisation is observed in lanes 1 – 11 in the high molecular weight range (including a distinct band at approx. 9.2 kb). This result is intriguing since exclusively prokaryotic sequences are to be detected by the applied probes. It is not clear why it was not possible to choose suitable hybridisation conditions which do not produce that immense background staining and why maize genomic sequences should produce hybridisation signals of that substantial kind with prokaryotic DNA. 3/ Comparing lane 4 with lanes 5 – 11, a hybridisation signal corresponding to the 6.1 kb fragment is clearly visible in all tested samples indicating the presence of vector backbone sequences at low concentrations. The notifier's conclusion that "MON 87460 DNA from seven generations showed no detectable hybridisation signals,… and do not contain any detectable backbone sequence from the transformation vector PV-ZMAP595" is not supported by the gel photo and thus invalid. Please provide a conclusive Southern blot in support of the hypothesis. [Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D. (2007). Molecular analysis of corn MON 87460. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	1/ On the request of the EFSA GMO Panel the applicant provided additional Southern analyses clearly showing the absence of vector backbone sequences (additional information October 2010). 2/ The Southern blot displayed in Figure 15 has the purpose to show that the insert is stable. Although there is a quite strong background both expected bands can be clearly seen in all generations. Therefore, this blot can be used to show stability of the insert. 3/ The applicant has provided Southern analyses showing absence of vector backbone sequences using separate probes (Song 2010). The band of approximately 6.1kb can also be seen in the sample with the conventional maize therefore it is confirmed this is unspecific hybridisation. Song, Z., et al. (2010) Confirmation of the absence of plasmid vector PV-ZMAP595 backbone sequence in the genome of MON 87460 by Southern Blot analysis. Report RAR-10-282. Monsanto Co., St. Louis, USA [as part of confidential dossier information, additional information received on 4 October 2010].

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 02 Information on the sequences actually inserted or deleted	Federal Ministry of Health	Specific comments on study report MSL0020487 (Skipwith et al. 2007) (cont.): 4.5 PCR and DNA Sequence Analysis to Examine the MON 87460 Insertion Site: The notifier states that a PCR amplification across the whole transgenic insert (including maize genomic flanking sequences of the maize genome) has not been performed. The PCR amplification of 3.3 kb fragment (= length of the whole transgenic insert) is technically no problem and would have been a nice confirmation for the insert location and the arrangement of the genetic elements of the insert. Please provide an explanation why this PCR assay was not performed. [Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D. (2007). Molecular analysis of corn MON 87460. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	Southern analysis, sequencing and PCR amplification of the pre-insertion locus are judged sufficient by the EFSA GMO Panel to conclude on the insert location and the arrangement of the genetic elements of the insert.
Austria	D, 03 Information on the expression of the insert	Federal Ministry of Health	For the evaluation of the expression levels of the two transgenes in GM maize MON87460 the applicant provides data from field trials conducted in the US (Mozaffar and Silvanovich 2008) and Chile (Shi et al. 2008). In these studies, reference is made to the same production plans (06-01-B3-04 and 06-45-B3-02) as in the studies presented for the comparative assessment (see comment on Comparative Assessment). Although not explicitly stated by the notifier this suggests that the same field trials were used for the production of material, for the comparative assessment and for the assessment of expression levels. Additionally no data on expression levels of the single sites, but only pooled data were presented, except for one site in Chile We request that the notifier presents an analysis of expression data for the individual samples gained at the various locations and to submit the missing production plans. In addition, both field trials where conducted for one single season	In the report processed data have been reported but the raw non-pooled protein expression data can be found in Mozaffar and Silvanovich (2008b). The aim of the field trials for protein expression experiments is to gather information on the ranges of protein expression levels. The use of different sites exposes the plants to a range of environmental conditions. Therefore, these trials are considered sufficient by the EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			only. It was shown that the expression patterns can vary considerably between individual plants from different locations and from subsequent growing seasons (see e.g. Nguyen and Jehle 2007). In order to take into account the variability of biotic and abiotic factors we request that submitted expression data are established for more than one growing season at a specific site in order to address varying environmental conditions. If no such data are presented, a justification why data from one growing season would suffice should be given. [Mozaffar, S. and Silvanovich, A. (2008). Assessment of the CSPB and NPTII protein levels in tissues of drought tolerant corn MON 87460 produced in 2006 U.S. field trials. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Nguyen, H. T. and Jehle, J. A. (2007). Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. Journal of Plant Diseases and Protection 114(2): 82-87. Shi, L., Chinnadurai, P., McClain, J. S. and Silvanovich, A. (2008). Amended report for MSL0021185: Assessment of the CSPB and NPTII protein levels in tissues of drought tolerant corn MON 87460 produced in a 2006-2007 Chilean field trial under well-watered and water-limited conditions Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	
Austria	D, 03 Information on the expression of the insert	Federal Ministry of Health	D.3 (c) Expression of potential fusion proteins The assessment of potential fusion proteins is based on studies presenting a bioinformatics analysis of the sequences present in GM maize MON87460. These results are therefore theoretical in nature and not supported by experimental data to assess which sequences coding for potential fusion proteins are actually transcribed/translated in GM maize MON87460.	The definition of ORFs used by the EFSA GMO Panel implies that an insertion will always result in the identification of at least 12 ORFs. These ORFs are merely stretches of DNA between two stop codons and there is no indication they would be transcribed. In the unlikely event that the ORFs would be transcribed and translated, bioinformatic analysis indicated that their putative translation products have no

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			The notifier should elaborate on the rationale why only the junction sequences between inserted transgenic sequences and maize genomic sequences have been analysed and why no other data were submitted to underpin the conclusions.	similarity to any known toxins or allergens.
Austria	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Ministry of Health	Data concerning the abiotic stress tolerance of GM maize MON87460 have been established during experiments conducted in growth chambers, in the greenhouse, and in field trials (Eberle et al. 2008 a&b, Eberle et al. 2009, Whitsel 2008b, Luethy 2009, Chomet et al. 2008). Moreover specific assessments of volunteer potential (Whitsel 2008a), the survival outside of cultivation areas (Rosenbaum & Eberle 2008), pollen morphology & viability (Whitsel & Sammons 2008) as well as seed germination and survivability (Whitsel 2007) are presented. Results.The differences between the test line (GM maize MON87460) and the control line at the trials under normal and well-watered conditions were low. The differences measured under water limited conditions may be attributable to the genetic modification conferring drought tolerance, i.e. the drought stress tolerant GM maize showed trends towards a higher final stand count, more plant height, more ear height, and a higher yield per hectare.In one of the field sites investigated for survival of GM maize MON87460 outside of cultivation (Rosenbaum and Eberle 2008) statistical significant differences in two parameters assessed were detected between GM maize MON87460 and the non-GM control: "early stand count" and "final stand count". Counts for these parameters were both higher in GM maize MON87460 than in the control and also outside the ranges observed for the reference varieties. Nevertheless, these differences were not followed up, but dismissed based on the argument that these differences were not detected at other test sites. Since the sites chosen in this field trial	Rosenbaum and Eberle (2008) reported survival and seed set of maize MON 87460 at one site, but found that the seed produced and replacement value was within the range of reference varieties and that there was a replacement value well below 1, which means the population was declining. Thus, no enhanced survival characteristics are indicated that suggest that the GM maize would present an environmental or agronomic problem compared to non-GM maize. The EFSA GMO Panel considered it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting, indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g., Gruber et al., 2008).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			vary significantly regarding vegetation cover and climatic conditions as they are situated in different States of the US (Montana, Nebraska, Illinois, and Texas), this reasoning is not conclusive without further investigating the conditions which favored survival of GM maize MON87460 in Montana. Regarding seed production the notifier calculated replacement values, the ratio between the numbers of seeds produced to the number of seeds sown. Since this was below 1 at the Montana site, the authors conclude that the population is declining. Although, in general, maize is not known to persist as a weed, in our view the differences detected in this study should be followed up as they might indicate implications for the outcrossing potential and thus risk management of GM maize MON87460. Moreover, two figures (Fig. 27 & 28) are missing in this Chapter of the Technical Dossier (p. 103 & p. 105). We request that the notifier provides these figures. [Rosenbaum, E. W. and Eberle, M. A. (2008). Assessment of survival of drought tolerant corn MON 87460 in unmanaged environments during 2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). The missing figures were provided as spontaneous submission by the applicant (letter dated 03/10/2012). Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17. Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594. Rosenbaum, E. W. and Eberle, M. A. (2008). Assessment of survival of drought tolerant corn MON 87460 in unmanaged environments during 2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.
Austria	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Ministry of Health	D.4.5 Evaluation of seed germination and dormancy: Specific comments on the report on dormancy and germination evaluation (Whitsel 2007): The control substance from one production site and several reference substances contained little amounts of GM maize MON87460 (see p. 11-12). For a certain comparison between conventional control maize and GMO maize, control and reference substances should be free of the test substance MON87460. Data are based on one season only. Statistical certainty should be guaranteed by repeating the experiments for at least one additional	The EFSA GMO Panel noted that the seed germination tests followed the AOSA protocol. Further, no enhanced dormancy was observed in the GM maize which would indicate increased weedy characteristics.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			year to account for environmental influence and to verify the current results, since for one location at 10°C a significant difference between test substance and control substance has been observed for total germinated and viable firm swollen seed. Also p-values of the comparison between the test substance and reference substance and across temperature regimes respectively have not been presented. No argumentation was done why GLP Standards were not required for this study. [Whitsel, J. E. (2007). Dormancy and germination evaluation of drought tolerant corn MON 87460 using seed produced at three U.S. sites during 2006. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	
Austria	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	Federal Ministry of Health	D.5. (a) Genetic stability of the insert in MON87460: Please see comments on Chapter D.2 "Specific comments on study report MSL0020487 (Skipwith et al. 2007)" [Skipwith, A., Feng, D., Groat, J. R., Tian, Q. and Masucci, J. D. (2007). Molecular analysis of corn MON 87460. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	See above.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 06 Any change to the ability of the GM plant to transfer genetic material to	Federal Ministry of Health	D.6 (a) Plant to bacteria gene transfer: Antibiotic resistance. GM maize MON87460 was produced employing an antibiotics resistance gene. The aminoglycoside antibiotics inactivated by nptII comprise kanamycin, neomycin and geneticin. At present EFSA uses the following arguments in favour of the use of ARM genes: 1) Marginal gene flow from plants to bacteria in natural environments;2) Resistance against outdated antibiotics;3) Existing high levels of aminoglycoside resistance in natural environments.This line of argumentation suffers several flaws as demonstrated in Wögerbauer (2007), where it was shown that low horizontal gene transfer rates are of little value assessing potentially adverse long term effects, that aminoglycoside antibiotics play their role in antimicrobial chemotherapy, albeit with country-specific preferences, and that the background levels of aminoglycoside resistances are highly variable and differ considerably from country to country and from species to species.To simplify safety assessments alternatives to ARM genes should be used (e.g. herbicide resistance genes or the PMI gene). Furthermore, after the selection process the ARM genes can be removed. The notifier must have had this in mind because he placed the nptII expression cassette between two loxP sites which can be used to excise the DNA sequences in between those two elements. However, this option was not exploited but rejected with the argument, that a lack of safety concerns around the NptII protein made it unnecessary to excise the nptII gene using the loxP sites that flank this gene (Technical Dossier, page 280). [Wögerbauer, M. (2007). Risk assessment of antibiotic resistance marker genes in genetically modified organisms. Forschungsberichte der Sektion IV. Vienna, BMGFJ. 5: 1-132.]	The EFSA GMO Panel concluded that adverse effects on human and animal health and the environment resulting from the transfer of the *npt*II and *csp*B genes present in maize MON 87460 to bacteria are unlikely, because of a highly limited potential for gene transfer. Taking into account the different exposure routes, this conclusion is mainly based on the following assessment: (1) the integration of the *npt*II and *csp*B genes through non-homologous recombination is most unlikely; (2) enhanced horizontal transfer of the *npt*II gene due to Cre-*lox* mediated recombination is unlikely; (3) the stabilisation of the *npt*II gene into bacterial cells by double homologous recombination of *A. tumefaciens* sequences flanking the *npt*II gene, and subsequent dissemination in the environment are unlikely; and (4) the unlikely but theoretically possible transfer of the *npt*II and *csp*B genes in maize MON 87460 to bacteria via gene replacement does not raise concerns due to the lack of an additional selective advantage which would be provided to the recipients in the receiving environments. The probability of horizontal gene transfer of the insert DNA of maize MON 87460 remains several orders of magnitude lower than the gene transfer efficiencies between bacteria. Therefore, its contribution (if any) to the environmental prevalence of *npt*II genes is negligible. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 07.01 Comparative assessment	Federal Ministry of Health	Data presented for the compositional analysis have been established in field trials in the US (Harrigan et al. 2008b) and Chile (Harrigan et al. 2008a). However, the production plans indicated in these studies 06-01-B3-04 ("Marcinkiewicz 2006") and 06-45-B3-02 ("Adu-Tutu 2008") are missing. Therefore, the management of the sites (e.g. herbicide use and fertilisation), sampling procedures and the conditions during the respective field trial cannot be assessed. We request that the notifier provides the production plans for the studies conducted for the comparative assessment. All field trials were conducted for one season only. However in order to cover variability of biotic (e.g. pest and disease pressure) and abiotic (e.g. weather conditions) factors, we recommend to include data on more than one season in the assessment or at least to present evidence that no unusual biotic or abiotic conditions prevailed during the respective season. In order to be able to assess whether the submitted data from field trials were adequate to address different environments and climatic conditions in a representative way, additional information on the assessed environments and the climatic conditions prevailing during the trials is necessary. We request that the notifier, furthermore, presents a justification for the selection of the locations chosen (e.g. agronomic description of the area) and a justification why data from one growing season are sufficient. Compositional analysis from the 2006 U.S. production (Harrigan et al. 2008b): 27 statistically significant differences (three from the combined-site analysis and 24 from the individual site analyses) were found. The combined site analyses showed higher contents of ash, stearic acid and eicosenoic acid in MON87460 compared to the control. All other statistically significant differences occurred at one site only,	The production plans for USA and Chilean trials (Mulesky, 2007; Adu-Tutu, 2008) were provided as part of the additional information supplied to EFSA in October 2010 *). Biotic and abiotic stressors were assessed in several field trials: eight locations in USA in 2006 [Sammons, 2009], 5 locations in USA in 2006 [Whitsel and Clark, 2009], 3 locations in Chile in 2006-2007 [Eberle, 2009a], 3 locations in USA in 2007 [Eberle, 2009b], two locations in USA in 2007 [Sammons, 2008], 10 locations in USA in 2007 [Rosenbaum, 2008]. *) Adu-Tutu, K., et al. (2008) Amended Report for MSL0021095: Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460, MON 87460 × NK603, MON 87460 × MON 89034 × NK603, and MON 87460 × MON 89034 × MON 88017 in Chile during 2006-2007. Production Plan #: 06-45-B3-02. Amended 1 - MSL0021759. Monsanto Co., St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012] Eberle, M. (2009a) Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 under well-watered and water-limited conditions in Chilean field trials during 2006-2007. Study number 07-01-B3-19. Report number MSL0021857. Monsanto Co., St. Louis, USA [as part of confidential dossier information Eberle, M. (2009b) Amended report for MSL0021856: Phenotypic evaluations and ecological interactions of

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			demonstrating a slightly different site adaptation of the test variants. [Harrigan, G. G., Miller, K. D. and Sorbet, R. (2008a). Amended report for MSL0021180: Compositional analyses of forage and grain collected from drought tolerant corn MON 87460 grown in a 2006/2007 Chile field production. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Harrigan, G. G., Miller, K. D. and Sorbet, R. (2008b). Compositional analyses of forage and grain collected from drought tolerant corn MON 87460 grown in a 2006 USA field production. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	drought tolerant corn MON 87460 Under well-watered and water-limited conditions in U.S. field trials during 2007. Study number REG-07-213. Report number MSL0022395. [as part of confidential dossier information Mulesky, M., et al. (2007) Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460 in the U.S. during 2006. Production Plan: 06-01-B3-04. MSL-0020810. Monsanto, St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012] Sammons, B., et al. (2008) Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 under well-watered and water-limited condition in U.S. field trials during 2007. Study number 07-01-B3-10. Report number MSL0021353. Monsanto Co., St. Louis, USA. [as part of confidential dossier information] Sammons, B., et al. (2009) Amended report for MSL0020951: Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 in irrigated U.S. field trials during 2006. Study number 06-01-B3-02. Report number MSL0022393. Monsanto Co., St. Louis, USA. [as part of confidential dossier information] Whitsel, J.E., Clark, P.L. (2008) Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 in U.S. field trials during 2006. Study number 07-01-B3-09. Report number MSL0021120. Monsanto Co., St. Louis, USA. [as part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 07.01 Comparative assessment	Federal Ministry of Health	Compositional analysis from the 2006/07 Chilean production (Alba et al. 2008; Harrigan et al. 2008): In the Chilean field trials, 2 different irrigation regimens were applied: a well-watered and a water-limited treatment. In the combined site analyses the well-watered MON87460 grain had significantly higher contents of total fat and Mg compared to the control. 12 significant differences were found in one or two locations. Supplementary analytes in well-watered and water-limited crops were investigated to reveal water stress induced composition differences. These were sugars and polyols, free proline, glycine betaine and choline, as well as metabolites that are generally associated with stress responses, such as salicylic acid and abscisic acid. At the fourth - separately analysed - production site (QUI) a disproportionately high number of significant differences (22 components, 8 metabolites) were revealed. According to Harrigan et al. the fourth site (QUI) did not meet the criteria that the commercial reference varieties had to exhibit concerning phenotypic responses appropriate to the intended treatment (Harrigan et al. 2008). The notifier should illustrate which criteria were applied (and not met) in that case and should explain the relevance of these findings in relation to the overall conclusions drawn from these field trials. The respective reference (Whitsel et al., 2008; MSL0021097) quoted in Harrigan et al. 2008a is missing. Please provide this crucial information. Under well-watered conditions, abscisic acid was found to be significantly different in the combined-site analysis as well as in one individual site analysis (site CL) of forage. Furthermore, three outliers (PRESS residual values higher than 6) were identified for this analyte (abscisic acid), of which only one was	With regard to the reference by Whitsel et al. (2008): The conditions pertinent to - and data generated from – the Chilean field trials in this study are described in the report by Eberle (2009a) The data in the reference Eberle (2009a; table 5, page 33) show that, in the location Quilota (QUI), the agronomic/phenotypic characteristics of the commercial varieties differed little between water-limited and well-watered conditions, unlike the bigger differences observed in the other three locations. The combined-site statistical analysis did not include data from the site QUI.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			considered a true outlier. This value of the test line (MON 87460, forage) at site CT, which PRESS residual value was > 12, was removed from further analysis. It is not quite clear why the other outliers which PRESS residual values lay outside of ± 6 were not considered true outliers and not removed. Furthermore, as removing data may influence the validity of the dataset, two statistical analyses (one with the outliers and one without) could have been performed. The notifier is thus asked to further explain his decision and the underlying concept regarding the outliers. The Chilean field trial design used only 4 sites; thus, it does not meet the new EFSA standards (EFSA 2009, Chapter 2.3.3), which require a minimum of eight sites. Therefore, this design seems limited as to demonstrating whether the GM plant is different from its appropriate conventional counterpart and/or equivalent to commercial varieties, apart from the inserted trait(s). For all these reasons, the statistically significant differences as for abscisic acid should be under special consideration, particularly in respect of a possible (site-related) correlation between the introduced trait and the observations made. [Alba, R. M., Miller, K. D. and Sorbet, R. (2008). Amendment 1 of report for MSL0021549: Metabolite analyses of forage and grain collected from drought tolerant corn MON 87460 grown in a 2006/2007 Chile field production. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. EFSA (2009). Scientific opinion of the GMO Panel on statistical considerations for the safety evaluation of GMOs. The EFSA Journal 1250: 1-62.	

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			Harrigan, G. G., Miller, K. D. and Sorbet, R. (2008). Amended report for MSL0021180: Compositional analyses of forage and grain collected from drought tolerant corn MON 87460 grown in a 2006/2007 Chile field production. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	
Austria	D, 07.04 Agronomic traits	Federal Ministry of Health	Data regarding the agronomic assessment is presented under D.4 in this notification and is based on five studies conducted in the US (Rosenbaum et al. 2008; Sammons et al. 2008; Whitsel and Clark 2008; Eberle 2009a; Sammons et al. 2009) and one study conducted in Chile (Eberle 2009b). The Chilean study (Eberle 2009b) and two US studies (Sammons et al. 2008; Eberle 2009a) were conducted under water-limited conditions as well as under conditions where sufficient water was provided for cultivation of maize. In two studies (Eberle 2009a; Eberle 2009b) production plans (06-45-B3-02, 07-01-B3-03) are mentioned, but not attached to the notification. In other studies (Rosenbaum et al. 2008; Sammons et al. 2008; Sammons et al. 2009) no reference to production plans is made and the information on the trial sites is limited to planting information, soil description and cropping history. There the notifier states that "agronomic practices used to prepare and maintain each study site were characteristic of each respective region". In the study by Whitsel and Clark 2008, the applicant notes that "pesticides were applied to prevent the trials from being compromised". We request that the notifier submits the missing production plans and additional information on the agronomic practices applied in the trials in each respective region. Additionally information on the conditions in each of the respective geographic regions, and evidence concerning the representativeness of the chosen study sites should be submitted.[Eberle, M. A. (2009a). Amended report for MSL0021856: Phenotypic evaluations and ecological interactions of	The missing production plans were provided by the applicant as additional information (cf., letter dated 03/10/2012).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			drought tolerant corn MON 87460 under well-watered and water-limited conditions in U.S. field trials during 2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Eberle, M. A. (2009b). Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 under well-watered and water-limited conditions in Chilean field trials during 2006-2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Rosenbaum, E. W., McPherson, M. A., Clark, P. L. and Ahmad, A. (2008). Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 in well-watered U.S. field trials during 2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Sammons, B., Clark, P. L. and Ahmad, A. (2008). Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 under well-watered and water-limited condition in U.S. field trials during 2007. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Sammons, B., Clark, P. L. and Ahmad, A. (2009). Amended report for MSL0020951: Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 in irrigated U.S. field trials during 2006. Dossier EFSA/GMO/NL/2009/70, Monsanto Company. Whitsel, J. E. and Clark, P. L. (2008). Phenotypic evaluations and ecological interactions of drought tolerant corn MON 87460 in U.S. field trials during 2006. Dossier EFSA/GMO/NL/2009/70, Monsanto Company.]	
Austria	D, 07.06 Effect of the production and	Federal Ministry of Healt	This chapter mentions only some common considerations about processing of maize and tells nothing about experiences with MON87460.	The issue of processing is also discussed in the opinion. No indications were found that the processing of MON 87460 would lead to changed characteristics of the processed products as compared to conventional maize

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
	processing	h		
Austria	D, 07.07 Anticipated intake/extent of use	Federal Ministry of Health	On page 258, the term 'margins of exposure' is used. This wording should be used very carefully, because normally it is used to define the margin between exposure and a toxicological reference point calculated from a dose-response curve out of subchronic or chronic studies (mostly carcinogenicity studies) EFSA 2005; EFSA/WHO 2006. Therefore, this term should not be used in relation to the outcome of acute gavage studies in mice. This is important in order to avoid the misinterpretation that there is enough safety in relation to the intake without having conducted or taken into account subchronic or chronic studies [EFSA (2005). Opinion of the Scientific Committee to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA Journal 282: 1-31. EFSA/WHO (2006). EFSA Meeting Summary Report - International conference with support of ILSI Europe on RA of compounds that are both genotoxic and carcinogenic. Risk assessment of substances that are both genotoxic and carcinogenic. Brussels, EFSA.]	The EFSA GMO Panel agrees with Austria concerning the adequacy of the MoE approach based on data obtained from acute studies.
Austria	D, 07.08 Toxicology	Federal Ministry of Health	Equivalence testing: According to the Technical Dossier (p. 259), equivalence between the E. coli- and the MON 87460-produced proteins was established by four tests: 1) SDS-PAGE analysis to confirm equivalent molecular weight 2) Western blot analysis to confirm equivalent immunoreactivity; 3) Glycosylation analysis to confirm the presence or absence of covalently linked carbohydrates in both proteins; 4) A double-stranded DNA-destabilizing ("melting") assay to confirm equivalent functional activity. Concerning the assessment of the enzymatic activity of the CspB	Besides the analyses mentioned by the competent authority, also MALDI-TOF-MS of tryptic peptides of CspB has been carried out. The data for the comparison of both CspB proteins as summarized in the opinion are derived from two dossier studies (Burzio, 2008ac) and one study provided as additional information (Chandu, 2010).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			proteins (Tech. Dossier, p. 262), it would be interesting to know how assay variability (25%) was determined. Important additional data are also missing (single values, ranges, etc.). Therefore, we cannot support the conclusions drawn by the notifier that "these results clearly demonstrate that the CspB proteins derived from MON 87460 and E. coli have equivalent functional activities." In case a microbially produced protein is used instead of the plant protein, the EFSA Guidance document (EFSA 2006) requires "comparisons of the molecular weight, the isoelectric point, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity". The notifier is, therefore, kindly asked to provide a comparison of the whole amino acid sequence of both in any case small CspB proteins (E. Coli + plant protein) and not to submit data of an N-terminal sequence analysis of the plant-produced CspB protein only. [EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99: 1-100.]	
Austria	D, 07.08 Toxicology	Federal Ministry of Health	D.7.8.1 Safety assessment of newly expressed proteins: The safety assessment of CspB and nptII expressed in GM maize MON87460 is based on: • the natural presence of the transproteins in food and feed; • the global distribution and consumption of GM variants expressing the same transproteins individually or in combination. This statement may be replied as follows: Proteins expressed by artificially arranged and plant-codon adapted genes are not naturally occurring food ingredients. Furthermore, distribution figures are no	Post-market monitoring for food and feed safety of maize MON 87460 is not recommended in the EFSA GMO Panel Scientific Opinion given the lack of safety effects that would trigger such monitoring. The body weight changes in the acute toxicity study with CspB that the member state refers to were not statistically significant. The EFSA GMO Panel considers acute toxicity testing does not add value to the safety assessment. The performance of the in-vitro protease resistance and bioinformatics analysis of newly expressed proteins as part of potential toxicity testing is common

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			safety arguments. Since in many countries GM labelling is not compulsory, epidemiological studies concerning GM food cannot be performed to find if they play a part in the proven increase of diet-related chronic diseases and noncommunicable disorders (FAO/WHO 2003; WHO 2008). The argument that CSP proteins are present in dairy produce and the gastrointestinal flora (see Tech. Dossier, p. 275 ff.) can hardly be seen as proof of safety, as this reasoning does not use a quantitative, scientific approach. Rather an estimation of the actual amount of CSP protein that interacts with the human body at the cellular level may be appropriate for safety assessment. Such calculations however are not provided by the notifier. Thus, the history of safe use is based on debatable arguments and not on reproducible, scientifically based, data. Moreover, the notifier refers to an acute toxicity study in mice with the CspB in which allegedly no treatment-related effects were observed (Tech. Dossier, p. 283). However, the body weight changes showed clear differences between the test (CspB) and the control group (BSA), albeit not significant at $p < 0.05$. For day 0 to 7, males showed increases in body weight of 1.4 g (test) compared to 2.1 g (control), whereas the results for females were 0.6 (test) against 0.9 (control). Thus, body weight changes were 50% higher in the control groups. For day 7 to 14, a difference of 20% was still to be seen in males. Females however showed no differences for that time period. A closer look at the individual data reveals: 2 males showed decreased body weights (test) compared to none in the control group, whereas 5 females showed decreased body weights (test) compared to 2 in the control group. These results	practice and in line with international (Codex) and EFSA guidance. The issues that the member state's comment pertains to, are addressed in the EFSA GMO Panel Scientific Opinion, section 5.1.3.2.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			indicate that body weight changes could be treatment-related and the used test design (single dose) may not be an appropriate instrument to reveal the significance of those findings. Because of the remaining uncertainties, and because of the additionally submitted test results (in silico analysis, in vitro digestibility assay) are limited in terms of representativeness of complex in vivo cell systems (König et al. 2004), it is necessary, with respect to food safety standards, to test both transproteins for a longer period using repeated dose studies, increasing the chance of revealing possible long-term effects. At last, we would like the notifier to provide a good explanation why a single dose of 4.7 mg/kg body weight was considered appropriate for testing the CspB protein, whereas the nptII protein was tested in doses up to 5000 mg/kg body weight (see Tech. Doss., p. 282 ff). [FAO/WHO (2003). Diet, nutrition and the prevention of chronic diseases. WHO Tech Rep Ser. Geneva: 1-150. König, A., Cockburn, A., Crevel, R. W. R., Debruyne, E., Grafstroem, R., Hammerling, U., Kimber, I., Knudsen, I., Kuiper, H. A., Peijnenburg, A. A. C. M., Penninks, A. H., Poulsen, M., Schauzu, M. and Wal, J. M. (2004). Assessment of the safety of foods derived from genetically modified (GM) crops. Food and Chemical Toxicology 42(7): 1047-1088. WHO (2008). The world health report 2008 - Primary health care; now more than ever. Geneva, World Health Organization: 1-124.]	
Austria	D, 07.08 Toxicology	Federal Minis	D.7.8.4 Testing of the whole GM food/feed: The feeding study ([redacted]) was performed with 3 groups of laboratory rats, each group consisting of 20 males and 20 females.	Based on the molecular characterisation of the genetic modification and the comparative compositional and agronomic & phenotypic analysis, no safety studies with the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		try of Health	The diets contained 11% MON 87460 (group 1), 33% Mon 87460 (group 2) and 33% control maize (group 3). The only statistically significant differences that could be associated with certain medical conditions are higher alkaline phosphatase serum contents in the females in the 11% MON 87460 corn group. In the 90-day rat feeding experiment with NK603 this was also observed in females of the 11% corn group. The three other differences indicate better health conditions in the GM groups (lower serum sodium and lower aspartate aminotransferase in females in the 33% MON 87460 corn group as well as lower urine specific gravity in females in the 11% MON 87460 corn group). Therefore, it can be concluded that the administration of grain from MON 87460 to rats for at least 90 consecutive days at concentrations up to 33% in the diet had no negative effects on the growth or health of rats. But this conclusion does not exclude potential negative effects only revealed in times of high performance such as reproduction or health stress or long-term influences. As far as the significance of this 90-day study is concerned, another element of uncertainty is the low amount of the transprotein in the diet of the rats. According to the CspB protein levels chart (Tech. Doss., Table 8, p. 86), GM maize grain contains ~ 0.063 µg/g fw of CspB; and thus, the daily intake of the pure protein was about 1-2 µg/kg body weight (= 1-2 ppb). Only highly effective toxins would show effects at such low dose levels. [[redacted]. A 90-day feeding study in rats with drought tolerant corn: MON 87460. Dossier EFSA/GMO/NL/2009/70, WIL Research Laboratories, LLC.]	whole food/feed in animals are considered necessary for the evaluation (EFSA, 2011). Apart from this, the applicant has provided a 90-day feeding study with grain from maize MON 87460 and a conventional counterpart, which did not show indications of toxicity (see section 5.1.3.4 of the Opinion). Therefore, in accordance with the EFSA Guidance document no additional animal feeding studies are required (EFSA, 2011). With respect to the evaluation of the newly expressed proteins, it is referred to section 5.1.3.2 of the opinion EFSA (2011) Guidance for risk assessment of food and feed from genetically modified plants. EFSA J. 9(5): 2150 [37 pp.]. Available online at: http://www.efsa.europa.eu/en/efsajournal/pub/2150.htm

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Austria	D, 07.09 Allergenicity	Federal Ministry of Health	7.9.1. Assessment of allergenicity of the newly expressed proteins It may be reasonable carrying out not only sequence comparisons but also searches for similarities in structures by using new powerful bioinformatic search tools (Schein et al. 2007). [Schein, C. H., Ivanciuc, O. and Braun, W. (2007). Bioinformatics approaches to classifying allergens and predicting cross-reactivity. Immunol Allergy Clin North Am 27(1): 1-27.] 7.9.2. Assessment of allergenicity of the whole GM plant or crop: From our point of view, additional in vitro studies (e.g. immunoblotting) for testing the possibility of over expression of endogenous maize allergens, e.g. Zea m1, Zea m12, Zea m14 (see http://thinkwise.unl.edu/IUIS/search.php?allergensource=Maize&searchsource=Search) are recommended.	With regard to the allergenicity assessment of newly expressed proteins and whole maize MON 87460: see section 5.1.4 of the opinion. The EFSA GMO Panel is of the opinion that allergenicity assessment was performed according to applicable guidance document.
Austria	D, 07.10 Nutritional assessment of GM food/feed	Federal Ministry of Health	The broiler study ([redacted]) was introduced as a confirmatory feeding study with 11 treatments and a total number of 1100 birds (broiler chickens). The statistic evaluation was conducted by Monsanto Statistics Technology Center. Therefore, the study cannot be regarded as fully independent. Additionally, the following questions arise: "The diets were not expected to contain any known contaminants (…)" (see [redacted]). On which facts do this assumption rely? – No analysis was conducted on undesirable/prohibited substances apart from mycotoxins and pesticides. No daily weight gains were assessed; the birds were weighed on Day 0 and Day 42 only. No additional information about the maize breeds and their cultivation/origin has been submitted. Why have different p-values been used at the statistical evaluation for diet x sex interactions ($p < 0.15$) compared to the evaluation of other parameters ($p \geq 0.05$)?	The characteristics of the maize grains of test and control maize used for dietary preparation for the 90-day and chicken broiler studies and the resulting diets are provided in the appendices B-E on pages 451-483 of the report on the 90-day rat feeding study (WI-2007-064 2008) WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			The amount of copper in the diets of the control maize was 4 times higher than in diets of MON87460 and conventional maize grain (see [redacted], Appendix II, Table 4). The amount of copper of 64 mg/kg in complete feed for broilers exceeds the maximum content of 25 mg/kg complete feed (see Reg. (EC) 1334/2003) and might lead to specific nutritional effects. Please provide an explanation for the toleration of these high copper contents in the diet. A significant difference between broilers fed diets containing MON87460 and those fed diets containing conventional control corn was detected among male birds for percent breast weight (27.2% and 26.2% respectively) and percent thigh weight (16.6% and 17.2% respectively). Please provide an explanation for this effect. [[redacted]. Comparison of broiler performance and carcass parameters when fed diets containing MON 87460, control, or reference corn. Dossier EFSA/GMO/NL/2009/70, Colorado Quality Research, Inc.]	
Austria	D, 10.07 Effects on animal health	Federal Ministry of Health	D.9.7 Effects on animal health Further studies - also conducted with ruminants and pigs – should be carried out to give a full assessment on feeding maize MON87460.	In accordance with the EFSA GMO Panel guidance document, since no biologically relevant differences were identified in the composition of maize MON 87460 as compared with its conventional counterpart, studies regarding effects on ruminants and pigs are not required.
Austria	D, 12.03 General Surveillance of the impact of the GM plant	Federal Ministry of Health	D.11.4. General Surveillance for unanticipated adverse effects: The Environmental Monitoring plan states that the responsibilities for the General Surveillance of GM maize MON87460 are shared between the authorisation holder and third parties, such as operators involved in the import, handling and processing of viable GM maize MON87460 (e.g. traders, silo operators, processors). These operators, represented by trade associations and existing networks (e.g.	EFSA reiterates that monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gave its opinion on the scientific content of the monitoring plan provided by the applicant, and considered that the scope and reporting intervals of the monitoring plan provided by the applicant are in line with the intended

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			COCERAL, UNISTOCK, FEDIOL), are obliged to report any potential unanticipated adverse effect to the authorisation holder (technical dossier, p.324) and to "remind their member organisations and companies … to monitor for potential unanticipated adverse effects" (technical dossier, p.326). However these organisations and companies, necessarily also at national level, are not specified by the notifier. Thus it remains unclear who will conduct the monitoring in practice. The notifier has not selected other networks further down the food/feed production chain for General Surveillance, stating that these mostly use processed, non-viable material. However, environmental effects of food/feed processing and use of GM maize MON87460 must be taken into account according to Regulation (EC) 1829/2003 (Art. 5.5b and Art.17.5b). Since this maize is used for food/feed products for the surveillance of unanticipated effects on human and animal health respective medical or veterinary networks should be involved. The notifier however did not include any medical or veterinary associations. In addition, General Surveillance will be influenced by the availability, extent and composition of existing networks in EU Member States. The notifier should therefore provide an overview of the national organisations to be involved in each individual EU member state. It must be clear before placing on the market of GM maize MON78460 which organisation and companies will be involved and to which degree they will be involved. Furthermore the notifier should document the commitment of organisations which will be part of the surveillance network to actively take part in the monitoring and to assist the notifier in the identification and reporting of unanticipated adverse effects. In Annex I of the notification general principles the operators apply	uses of maize MON 87460.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			for all the commodities they deal with and additional information on the existing systems is provided. There also the areas where general surveillance should be principally carries out are identified as ports, silos and processing facilities (Annex I). The notifier states that "exposure to the receiving environment will be limited and can easily be controlled by …. manual or mechanical removal and the application of appropriate herbicides" (Annex I). As no clear responsibilities are assigned in this respect, it remains unclear who actually will be responsible for activities aimed at limiting the exposure of the environment to GM maize MON87460, e.g. clean up measures in the case of accidental spillage during loading and unloading. The description of the monitoring methodology is based on passively collecting information. Any information on which specific data need to be gathered and how is missing. The notifier merely states that operators will report any adverse effects directly (or via EuropaBio) to the authorisation holder (technical dossier, p.326). A more proactive approach of GS, including specific activities for monitoring grain loss at different locations (e.g. ports, silos, processing facilities), should also be employed by the notifier.	
Austria	D, 12.03 General Surveillance of the impact of the GM plant	Federal Ministry of Health	D.11.4. General surveillance for unanticipated adverse effects (cont.): Additionally Annex I states that "operators are exempted from any additional duty which is not foreseen by the hereunder recalled and collected legislation", listing the General Food Law (Regulation (EC) No 178/2002) and regulations on food and feed hygiene (Regulations (EC) No 582/2004 & No 183/2005). According to these regulations the operators are obliged to implement and maintain procedures based on HACCP principles. However the notifier fails to outline how these principles match with the requirements of an environmental monitoring plan of the GM maize or an adaptation of these	EFSA reiterates that monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gave its opinion on the scientific content of the monitoring plan provided by the applicant, and considered that the scope and reporting intervals of the monitoring plan provided by the applicant are in line with the intended uses of maize MON 87460. As the scope of the application EFSA-GMO-NL-2009-70 does not include cultivation, the environmental risk assessment is concerned with the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			procedures for the requirements of general surveillance of GMOs, which probably differ from e.g. the monitoring of hygienic standards. In conclusion the proposed monitoring plan seems short of addressing relevant questions for the general surveillance of human and animal health and cannot be regarded as sufficiently elaborated for the monitoring of accidental spillage of GM maize MON87460.	accidental release into the environment of viable grains of maize MON 87460 during transport and processing for food and feed uses, and with the exposure through manure and faeces from animals fed maize MON 87460 grains. The environmental risk assessment identified no potential adverse effects to the environment. The EFSA GMO Panel considered that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of conventional maize varieties. Therefore, the EFSA GMO Panel concluded that no case-specific monitoring is necessary.
Belgium	General comments	BAC	1/ To describe the different maize growth stages a code system is used. It would be informative to include a scheme depicting these different growth stages of maize, mentioning the different codes used in the sampling procedures applied in the dossier. 2/ The T-DNA inserted in MON87460 contains two loxP sites. â€œThe loxP sites were inserted to facilitate the potential excision of the nptII cassette, specifically using CRE recombinaseâ€• (citation from Pg 36, second paragraph). The use of the Cre/Lox marker removal system is not explained in detail and only a single reference is given (Russell et al. 1992). Given that MON87460 is among the first dossiers that also includes such sequences (other example is LY058 = EFSA-GMO-NL-2006-31), a more detailed description and discussion on this topic, documented with update references seems relevant. 3/ There are many mistakes in the dossier: missing or incorrect references, missing figures (see comments under respective	1/ The codes for the different maize growth stages used by the applicant are well recognised and described in various text books. 2/ Upon request of the EFSA GMO Panel, the applicant provided on 04/10/2010 and 30/04/2012 additional information relevant for the risk assessment of the *lox*P sites present within the insert. This information contained multiple references related to the Cre-lox system. In addition, the EFSA GMO Panel has listed in its Scientific Opinion multiple references related to the Cre-*lox* system. 3/ The missing figures were provided as spontaneous submission by the applicant (cf., letter dated 03/10/2012).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			references).	
Belgium	C. Information relating to the genetic modification	BAC	Technical dossier, Part I, Pg 29, first paragraph: It is mentioned that the Agrobacterium strain used for transformation contains a disarmed Ti plasmid. However, it is not explained in detail how this was done, only the brief statement 'due to deletion' was included in the sentence. Perhaps a more detailed explanation can be included for reasons of completeness of the provided information.	The *Agrobacterium tumefaciens* is a widely used strain which contains a disarmed T-DNA. As indicated in the technical dossier detailed information can be found in Koncz and Schell (1986). Koncz, C., Schell, J. (1986) The promoter of T_L-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of Agrobacterium binary vector. Mol. Gen. Genet. 204: 383-396.
Belgium	D, 01 Description of the trait(s) and characteristics which have been introduced	BAC	1/ Technical dossier, Part I, Pg 33: For all genetic elements used in the transformation vector a reference is given, however only for one element also (aadA) the GenBank accession code is provided. For reasons of completeness this could also be done for the other genetic elements of the vector. 2/ Technical dossier, Part I, Pg 37, Fig. 3: Suggestion linked to previous remark: include GenBank accession code of original CspB protein in legend of Fig. 3 and indicate difference with codon optimized CspB L2V sequence by indicating V in bold. The legend could use the suggested name CspB L2V instead of CspB as such to stress the codon optimization. 3/ Technical dossier, Part I, Pg 39, one but last paragraph: The text mentions that the Csp protein in MON87640 consists of 66 amino acids while both Fig. 3 and Fig. 5 (= Fig. 37) show 67 amino acids. Is this just a typing error? 4/ Technical dossier, Part I, Pg 43, Figure 6, part B: The legend describes that increasing amounts of BSA are used while only one	1/ References have been provided for all elements. The GenBank accession code is not necessary for risk assessment. 2/ The legend is clear and complete and is considered sufficient by the EFSA GMO Panel. The L2V change is not the result of codon optimisation. 3/ The EFSA GMO Panel acknowledged this observation. This will not affect the outcome of the risk assessment as all analyses were performed with the correct sequence having 67 amino acids (Figure 3 and 5). 4/ The EFSA GMO Panel acknowledged this observation. The design of the experiment is considered appropriate by the EFSA GMO Panel. 5/ The EFSA GMO Panel acknowledged this observation. This will not affect the outcome of the risk assessment.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			amount (50 µg) is shown on the figure. 5/ Editorial comments: - Just a little mistake in the text concerning "1.1. Characterization of the CspB protein and its function", bottom of page 39: reference is made to figure 37, but it should be read figure 5 instead.- Technical dossier, Part I, Pg 39, last paragraph: This paragraph refers to Fig. 37 to illustrate the presence of RNP motifs in the AA sequence. However, Fig. 5 is identical to Fig. 37 and incorporated in the document on pg. 40, while Fig. 37 is found on pg. 273 (Section 7.8 Toxicology).	
Belgium	D, 02 Information on the sequences actually inserted or deleted	BAC	Technical dossier, Part I, Pg 79 – Bioinformatic analyses of MON87460 flanking sequences. BLASTn and BLASTx evaluations indicate that it is unlikely that the inserted T-DNA disrupted endogenous maize genes within the genomic DNA flanking the insertion. Nothing is mentioned however about an analysis to evaluate the presence and functionality of possible novel chimaeric ORF, as requested by "Guidelines for Molecular Characterization of Genetically Modified Higher Plants to be Placed on the Market" from WIV-SBB, Final version Feb 18, 2003 .	The analysis of ORFs is described under section D3(e) (page 95) and in study by Silvanovich and Tu (2009). Silvanovich, A., Tu, H. (2009) Updated bioinformatics evaluation of DNA sequences flanking the 5′ and 3′ junctions of inserted DNA in MON 87460: Assessment of putative polypeptides utilizing the AD_2009, TOX_2009 and PRT_2009 databases. Study number RAR-09-440. Monsanto Co., St. Louis, USA [as part of confidential dossier information]
Belgium	D, 03 Information on the expression of the insert	BAC	Technical dossier, Part I, Pg 93 Table 8 & 12 vs. Table 10 – A two-fold difference in accumulation level of the CspB protein was detected in the pollen samples collected at the US trial sites + Chilean QUI site versus the Chilean CT, CL and LUM sites. What could be the possible explanation? This is not discussed in this section.	It should be noted that differences in expression levels of newly expressed proteins between different field experiments are not uncommon.
Belgium	D, 04 Information on how the GM	BAC	Editorial comments: Technical dossier, Part I, Pg 104, Fig. 28. This figure is missing. Technical dossier, Part I, Pg 103, Fig. 27. This figure is missing.	The missing figures were provided as spontaneous submission by the applicant (cf., letter dated 03/10/2012).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
	plant differs from the recipient plant in:			
Belgium	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	BAC	- Technical dossier, Part I, Pg 151: Generational stability of the insert. The T-DNA inserted in MON87460 has two loxP sites. The loxP sites were inserted to facilitate the potential excision of the nptII cassette, specifically using CRE recombinase (cfr. Pg 36, second paragraph). From the Southern blot shown in Fig. 155 it is clear that the intact nptII cassette is present in all generations. Indirectly it can be concluded that no non-specific endogeneous recombinase activity could be detected causing an unexpected removal of the nptII cassette (during the breeding process). This aspect is not discussed in detail in the text and perhaps disserves some attention underlining the safe use of loxP sequences. In addition some scientific references (also elsewhere in the dossier) should be included illustrating the safe use of the Cre/Lox system in GM plants. - Technical dossier, Part I, Pg 157: The Invader® assay was used to select a homozygous plants. The principle of the assay is described briefly, but the result of the assay is not documented nor discussed. - Technical dossier, Part I, Pg 157 and Table 44, Pg 158: It is mentioned that the segregation patterns reported in Table 44 are based on PCR-based assays. The header of Table 44 mentions that the table shows data of "segregation patterns of cspB between generations of Mon8746" and here a reference is mentioned. No further explanation is given. More detailed info would be welcome here.	Stability of the MON 87460 insert has been shown and stability of lox cassettes has been shown previously by Dale and Ow (1990, Gene vol. 91:79). The EFSA GMO Panel has requested further information concerning the presence of the *npt*II gene and the possibility for horizontal gene transfer. Please see the scientific opinion for further details. Dale EC and Ow DW, 1990. INTRAMOLECULAR AND INTERMOLECULAR SITE-SPECIFIC RECOMBINATION IN PLANT-CELLS MEDIATED BY BACTERIOPHAGE-P1 RECOMBINASE. Gene, 91, 79-85.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Belgium	D, 07.03 Selection of compounds for analysis	BAC	1/ The expert agrees with the conclusion of the applicant. The file contains convincing evidence that maize MON87460 is compositionally equivalent to commercial maize. Nutrients, anti-nutrients and secondary metabolites were selected according to up to date knowledge. The applicants refers to the OECD document to motivate the selection of compounds. Comment 1: We repeat our previous comment that this OECD document needs to be updated at least in the field of fibre particularly in view of the ongoing discussions on the role of fibre constituents in human nutrition, among others the definition of dietary fibre and the definition of whole grain products. 2/ Comment 2: Is there any information on the resistance to mould development and mycotoxin formation of maize MON87460 in comparison to commercial maize? Compositional equivalence is always studied, in this type of applications, in terms of nutrient, anti-nutrients and particular secondary metabolites. Taking into account the importance of mycotoxins in human and animal health, these range of compounds definitely need more attention in future applications.	1/ The EFSA GMO Panel agrees with the Belgium on the important role of the OECD consensus documents and the work currently being carried out by the OECD Task Force to revise and update these documents. 2/ Compositional (including pesticides) and mycotoxin analyses were performed in grain that was used to formulate diets for the broiler chicken feeding studies [study report MSL0021408, 2008]. The same lot of grain was used also to prepare diets for the 90-day rat feeding study [study report WI-2007-064, 2008] WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Belgium	D, 07.07 Anticipated intake/extent of use	BAC	This section is well-documented. Most conservative intake calculations were made, providing conservative high end exposure scenarios. Protein levels were taken from the CBI Mozaffar and Silvanovich study (2008a): field trail US 2006 season, grown under normal agronomic practices. This study provided the highest protein levels (Chile 2006-2007: lower protein levels, grown under well-watered and water-limited conditions). For the estimation of the animal dietary intake, the mean protein levels used were expressed on a dry weight basis, however for the human dietary intake mean protein levels were expressed on a fresh weight basis. What is the reasoning for using fresh weight figures? (However this has no consequences on the outcome of the Margin of Exposure)	Protein levels for all tissue types were calculated on a microgram (µg) per gram (g) fresh weight (fwt) basis. Moisture content was measured for all tissue types and both protein levels were also expressed as the mass of protein per dry weight (dwt) of tissue [Mozaffar, 2008]. Presumably the applicant used fresh weight for human food so as to be able to be able to use intake data on maize-based food products (wet weight of maize flour, popcorn, sweet corn) Mozaffar, S., Silvanovich, A. (2008ab) Assessment of the CSPB and NPTII protein levels in tissues of drought tolerant corn MON 87460 produced in 2006 U.S. field trials. Monsanto Technical Report MSL0021033. Monsanto Co., St. Louis, USA [as part of confidential dossier information]
Belgium	D, 07.08 Toxicology	BAC	Comment 1 The compositional analysis was already extended with a more targeted screening on additional secondary metabolites considered to be associated with stress tolerance. However, stress-associated metabolism is very complex and not well understood yet. In our opinion this type of modification also triggers the testing of the MON87460 grain both in the 42-day feeding study in broilers AND in the 90-day oral toxicity study in rats. Both tests were performed in this application. Comment 2 The trials with rats and mice have one common shortcoming, i.e. the power of the statistical analysis is too low in order to able detected significant differences, if present, because he number of animals per treatment is too small.	The Panel thanks Belgium for its insightful comments. The animal trial design was based on OECD technical guideline 408 (for chemical toxicity testing in rodents during 90 days), which in this case was adapted to testing a whole food/feed product. The size of each treatment group consisted of 20 animals / gender / treatment.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Belgium	D, 07.09 Allergenicity	BAC	Comment 1: it is now well established that provided antigens are formulated in combination with potent adjuvants or when antigen exposure coincides with a condition of acute immune (hyper)reactivity in genetically predisposed individuals, immune tolerance of the organism to any environmental food or airborne antigen can be overcome, resulting in allergic sensitisation and disease. Notwithstanding such extreme conditions, environmental antigens differ dramatically in their capacity to elicit allergic sensitisation under conventional conditions. As common traits, allergenic antigens share physicochemical properties such as high solubility and resistance to proteolysis but also the presence of enzymatic activity, especially protease and lipase activity (Mills et al, Crit Rev Food Sci Nutr 44:379-407, 2004; Shakib et al, Trends Immunol 29: 633-642, 2008). Accordingly, the present assessment of allergenic potential of the heterologous CspB and NptII proteins expressed in the MON87460 maize GMO was performed taking into account these criteria in addition to the source of the protein, amino acid sequence similarity to known allergens and allergen peptides, and susceptibility to digestive proteolysis. Mills EN, Jenkins JA, Alcocer MJ, Shewry PR. 2004. Structural, biological, and evolutionary relationships of plant food allergens sensitizing via the gastrointestinal tract. Crit Rev Food Sci Nutr 44: 379-407. Shakib F, Ghaemmaghami AM, Sewell HF. 2008. The molecular basis of allergenicity. Trends Immunol 29: 633-642	The EFSA GMO Panel appreciated this comment of Belgium.
Belgium	D, 07.09 Allergenicity	BAC	Comment 2 :Allergenic potential of CspB: - Protein function: The protein is characterized in literature as a small protein containing a RNA-binding sequence and exerting a melting or unwinding activity on polynucleotides such as single stranded and	To request an additional pepsin-resistance test with RNA-bound CspB is considered unlikely to add much to the data already provided Under the conditions of pepsin resistance tests (low pH), the RNA-protein binding is likely to be

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			double stranded RNA. > low risk. - Source: Bacillus subtilis >low risk. - Expression: The protein is present in grain although reportedly at low levels. Dose-response characteristics of allergen exposures are especially difficult to predict and the underlying molecular mechanisms still elusive. This means that the presence of a protein rather than its expression level is to be considered. > potential risk. - Sequence similarity: A bioinformatics screen was performed > low risk. - Susceptibility to (digestive) proteolysis: Sequential exposure to acid pH/pepsin and neutral pH/pancreatin revealed rapid degradation of the protein into a smaller fragment (acid pH/pepsin) and the further degradation of this fragment under neutral pH/pancreatin. The results reported are convincing but a few questions remain. First, the source of the recombinant CspB used in these assays is not specified nor whether the wild type or the MON87640 mutant (L2V) was used. Second, these assays were performed with free CspB, not bound to RNA. While it is likely that in the maize plant free CspB will be present, also CspB bound to RNA should occur. Quoting from the report 'In the absence of polynucleic acids, the CspB protein has a very low thermodynamic stability and is susceptible to rapid proteolytic degradation' (page 39), it seems crucial to determine the sensitivity to proteolysis also of the RNA complexed CspB in order to fully judge this characteristic. > awaiting further info and data. Conclusion: Monsanto performed a thorough analysis of characteristics associated with allergenicity. The results are satisfactory but before reaching a final conclusion additional data on the susceptibility to proteolysis are necessary.	implicated by RNA protonation (positive charge). In addition, RNA will be prone to certain chemical reactions at pH<2 and therefore less stable. With regard to RNA stabilization at higher pH values, Schindler et al. (1999) studied trypsin-mediated degradation of CspB and CspB bound to RNA at physiological pH values (e.g. Schindler et al., 1999; http://hwmaint.jbc.org/cgi/content/full/274/6/3407). While the CspB bound to RNA was less rapidly degraded than CspB alone, the results from the study by Schindler et al. show that under influence of trypsin, more than 80% of CspB has still been degraded at pH 7 after 120 minutes. Trypsin digestion is not the primary test of the degradation assays recommended by guidance (e.g. EFSA, Codex). . Codex alimentarius (2003) Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003. Joint FAO/WHO Food Standards Programme, Rome and Geneva. Available online at: http://www.codexalimentarius.org/download/standards/10021/CXG_045e.pdf EFSA (2011) Guidance for risk assessment of food and feed from genetically modified plants. EFSA J. 9(5): 2150 [37 pp.]. Available online at: http://www.efsa.europa.eu/en/efsajournal/pub/2150.htm Schindler, T., et al. (1999) The family of cold shock proteins of Bacillus subtilis. J. Biol. Chem, 274(6): 3407-3413. Available online at: http://hwmaint.jbc.org/cgi/content/full/274/6/3407

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Belgium	D, 07.09 Allergenicity	BAC	Comment 3: Allergenic potential of NptII: - Protein function: >low risk - Source: A non-virulent strain of E.coli > low risk - Expression: > reduced risk - Sequence similarity: > low risk - Susceptibility to (digestive) proteolysis: > low risk Conclusion: Monsanto performed a thorough analysis of characteristics associated with allergenicity. The results do not point to a significant risk for allergenicity of the NptII protein in MON87460 maize grain when used as feed or food.	The EFSA GMO Panel appreciated the allergenicity assessment of NptII protein by Belgium.
Belgium	D, 07.10 Nutritional assessment of GM food/feed	BAC	Four studies with animals were reported, one on the performance of broilers, one on health aspects of rats, and two on health aspects of mice. An important drawback in the broiler trial is, that data were collected on pen level instead of animal level, so that the most important information on variability within the data is lost. Nevertheless, the number of pens is sufficient to detect significant differences if present, but that could have been different, if data were collected at animal level. Another important aspect is the mortality rate, which is much higher than within farming conditions.	The EFSA GMO Panel agrees that analyses of data collected at the individual bird level would add value to the broiler feeding study. However, since no adverse effects were found, these analyses are not considered essential by the EFSA GMO Panel.
Denmark	General comments	Danish Environmental Protection Agency	Denmark finds it appropriate to ask the applicant why the nptII-gene has not been removed as it in our view would be possible. Denmark can not accept GMO's with nptII-genes and ARM's in general.	Not in the remit of the EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Finland	D, 10.01 Persistence and invasiveness	Board for Gene Technology	The Board for Gene Technology is concerned about the scientific quality of the ERA especially regarding the study in Part I, Technical dossier, D, 4.3 (d). The applicant has not adequately assessed the potential for the drought tolerant maize to have greater persistence or invasiveness in the environment compared to conventional maize (see e.g. Nickson 2008). According to Nickson, it is possible to study these phenomena in field studies assessing survival and competition in non-crop environment. The application contains the results of one study by Rosenbaum and Eberle that assesses the abil-ity of MON 87460 maize to survive in unmanaged environment compared to conventional corn control. According to the application, only early stand count and final stand count were greater for the MON 87460 when compared to the control. Furthermore, the replacement value was much less than one, which is interpreted to mean that the population is declining. Thus the draught tolerance trait confers no biologically meaningful change to the fitness, invasiveness, or potential for maize to persist outside of managed agricultural environments. However, the results of Rosenbaum and Eberle are based on very small research material and one growing season only (e.g. the replacement value is based on one site only) and the results were not analysed statistically. Our point of view is that no definite conclusions of the change of fitness or invasiveness of the MON 87460 maize, or potential for the maize to persist outside of managed agricultural environ-ments can be made based on the above mentioned field study.	The EFSA GMO Panel refers to the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. In the present case, the EFSA GMO Panel considered the persistence and invasiveness studies sufficient. Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable grains from maize MON 87460 during transport and processing. The EFSA GMO Panel considered it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting, indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g.,

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
				Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Survival of maize plants outside cultivation in Europe is mainly limited by: a combination of low competitiveness; absence of a dormancy phase; and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MON 87460, drought tolerance alone is not likely to provide a selective advantage outside cultivation in Europe. Therefore, the EFSA GMO Panel considered it very unlikely that maize MON 87460 will differ from conventional maize varieties in their ability to survive until subsequent seasons, or to establish feral populations under European environmental conditions. Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17. Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.
France	General comments	Ministère de l'Economie (Consom	1/ Conclusion of the French Food Safety AgencyMaize MON87460 expresses the proteins CspB and NPTII. CspB allows the loss in yield under conditions of moderate drought stress to be reduced. NPTII has been used as a selection marker. The level of information submitted in the dossier on the molecular characterisation of the transformation event MON87460 is satisfactory. 2/ The results of the analysis of the chemical composition of the	1/ The EFSA GMO Panel considered that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants. 2/ The production plans for USA and Chilean trials (Mulesky, 2007; Adu-Tutu, 2008) were provided as part of the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		matio n)	forage and grain of maize MON87460 demonstrate the absence of any appreciable differences from the control maize with regard to the analysed compounds. The same is true of the nutritional qualities of this maize compared with those of the controls. As regards the toxicological evaluation of maize MON87460, the dossier contains studies of the administration purely of the newly expressed protein CpsB (4.7 mg/kg) and NPTII (5000 mg/kg). However, the dose of protein CspB evaluated in the study is inadequate and incompatible with the objectives of such a study. A new study with a higher dose, in accordance with OECD guideline 420, needs to be submitted. With regard to the study of the repeat administration of maize grain added to the feed (11 and 33%) for 90 days in the rat, the applicant must submit information on the cultivation conditions of the maize used. As a consequence, the French Food Safety Agency is unable to reach a decision on the safety of maize carrying the transformation event MON87460, of its grain and of products derived from it.	additional information supplied to EFSA in October 2010 The characteristics of the maize grains of test and control maize used for dietary preparation and the resulting diets are provided in the appendices B-E on pages 451-483 of the report on the 90-day rat feeding study (WI-2007-064 2008) Adu-Tutu, K., et al. (2008) Amended Report for MSL0021095: Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460, MON 87460 × NK603, MON 87460 × MON 89034 × NK603, and MON 87460 × MON 89034 × MON 88017 in Chile during 2006-2007. Production Plan #: 06-45-B3-02. Amended 1 - MSL0021759. Monsanto Co., St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012] Mulesky, M., et al. (2007) Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460 in the U.S. during 2006. Production Plan: 06-01-B3-04. MSL-0020810. Monsanto, St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012] WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]
France	A. General information	Ministère de l'Eco	(A) General information This application is a first application for the marketing authorisation of maize MON87460 genetically modified to reduce losses of yield caused by drought, for the importation and use of the maize plant as	A general comment is made by France; no specific question is posed to the EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		nomie (Consommation)	forage in animal feed and of the grain and derived products in human and animal food products. Drought is a major cause of losses in yield. In North America, it is estimated that 40% of annual losses are caused by restricted water resources. This is the first maize presenting this agronomic characteristic. The application does not concern its cultivation in the European Union. Maize MON87460 expresses a new protein, cold shock protein B (CspB). This brings a reduction in the loss in yield of this maize compared with a conventional maize when cultivated under restricted water conditions. Under normal irrigation conditions, the grain yield for MON87460 is equivalent to that of a conventional maize. In severe drought conditions, maize MON87460 is still subject to losses in yield. The gene inserted in maize MON87460 is taken from *Bacillus subtilis* and codes the protein CspB, which is known to promote the adaptation of this bacterium to environmental stress. CspB interacts with and suppresses secondary RNA structures, thereby promoting translation. Under drought stress conditions, CspB helps preserve normal cell function, facilitating the RNA translation involved in all plant functions: photosynthesis, stomatal conductance and carbon fixation. Finally, expression of the protein Csp improves the yield of the maize grain. Maize MON87460 also expresses the protein neomycin phosphotransferase II (NPTII). This protein is used as a selection marker. Protein NptII is able to inactivate aminoglycoside antibiotics such as neomycin and kanamycin.	
France	D, 07.01 Comparative assessment	Ministère de l'Eco	(7.1.3) Analysis of the chemical composition The comparative analysis of composition concerns the forage and grain obtained from two field studies: 1) maize cultivated at six different sites in the United States during the 2006 season and 2)	The EFSA GMO Panel appreciated this concise summary of the compositional analysis.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
	(1)	nomie (Consommation)	maize cultivated at four different sites in Chile in 2006-2007. The maizes cultivated in the two studies are different hybrids (LH59R3xLH244 for the USA and LH244xLH59 for Chile), with the maize hybrids carrying the event MON87460 compared with hybrids of the same cross that do not carry the transformation event. The maizes were cultivated alongside three (EU) or four (Chile) commercial, nontransgenic maize hybrids, which varied from one site to the next. The crops were raised under normal irrigation conditions in the United States and Chile and under restricted irrigation conditions in Chile. For each watering regimen, the composition of the MON87460 forage and grain was compared by variance analysis ($p < 0.05$) with a quasi-isogenic control for all sites taken together or site by site for the two watering regimens. One of the Chilean study sites was omitted from the overall analysis of the four Chilean sites because the agronomic data showed an unsatisfactory response as regards the different watering regimens. The data obtained for the commercial hybrids were used to establish the confidence intervals of each compound analysed. The data are also compared with literature data and data from the ILSI Crop Composition database. The analysis follows the OECD recommendations, covering 9 compounds from the forage and 68 from the grain, including antigrowth factors (phytic acid, raffinose, ferulic acid and coumaric acid). It also includes supplementary data on a number of secondary metabolites determined in the grain that are considered to be associated with drought stress (sucrose, glucose, fructose, glycerol, proline, glycine betaine, choline, abscisic acid and salicylic acid). Results of the studies under normal irrigation conditions The chemical composition of the forage and grain of maize MON87460 under standard cultivation conditions shows some	

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			significant differences from that of the control maize and the commercial varieties. However, the values remain within the confidence interval calculated from the data for the commercial hybrids. Similarly, for all but a few compounds the analysis results do not reveal any significant difference in the measured contents in the forage and grain of the osmoprotectant metabolites associated with drought stress. However, the measured contents are in all cases within the confidence interval determined for the commercial hybrids.	
France	D, 07.01 Comparative assessment (2)	Ministère de l'Economie (Consommation)	Results of the studies under restricted irrigation conditions The results are similar for the chemical compounds normally determined in this type of study and for the osmoprotectant metabolites associated with drought stress. The chemical composition of the forage and grain of maize MON87460 does not differ from that of the quasi-isogenic control maize under restricted-water conditions. It would have been interesting to carry out a multifactorial statistical analysis to compare the genotype interaction (MON87460 or isogenic) with the application of stress.	The interaction between genotype and the application of stress has been embedded in the mixed model analysis of variance for compositional data, denoted as TSjk = treatment by substance interaction effect. For details on statistical methodology see section 8.2 of the [Alba et al, 2008].
France	D, 07.08 Toxicology	Ministère de l'Economie (Consommation)	(7.8) Evaluation of the safety of proteins CspB and NptII The safety of protein CspB is based on the following data: The donor organism is Bacillus subtilis, from which a number of enzymes are approved for use in the food-processing industry. Bacillus subtilis is also sold as a probiotic in many parts of the world, including Europe. · Protein CspB belongs to the family of cold-shock proteins (CSD), which have a highly conserved CSD domain. This family is very widespread in living organisms that are, moreover, commonly found in the human digestive tract. The principal sources are dairy products (yoghurt), wheat and rice. A database search revealed that protein CspB shows sequence	The EFSA GMO Panel appreciates this concise summary.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			homology (35-98.5%) with natural, CSD-containing proteins found in various plant species or in microorganisms used in the dairy industry. An in-silico study demonstrated the absence of sequence homology between protein CspB and proteins toxic to humans or animals listed in current databases. The rapid breakdown in vitro of protein CpsB in simulated gastric and intestinal fluid (cf 7.9 Allergenicity).	
France	D, 07.08 Toxicology	Ministère de l'Economie (Consommation)	7.8.4) Subchronic toxicity study: A 90-day subchronic toxicity study has been carried out in the rat according to a protocol complying with international guidelines (OECD 408) and Good Laboratory Practice. Three groups of twenty animals per sex were given a feed containing 33% or 11% of maize MON87460 grain or a quasi-isogenic control maize. The chemical composition of the grain and of the diets was determined and their analysis did not reveal any significant difference.Statistical analysis (ANOVA) of the data for this study reveals some significant differences, in certain blood and urine parameters and in organ weights, between the rats fed the genetically modified maize and those fed under the same conditions with the control maize. However, these differences are within the range of values observed in the submitted historical data and are not significant from a toxicological viewpoint. The other parameters monitored in the study (growth, feed consumption, clinical observations, ophthalmological examination, behaviour, macro- and microscopic clinical pathology of the organs at the end of the study) did not show any significant differences between the groups. Based on these results, it can be concluded that the administration in the feed of genetically modified maize MON87460 to rats for 90 days does not cause any adverse effect in the animals. The highest dose is equivalent to 23.6 g/kg/day for males and 28.2 g/kg/day for females.	The EFSA GMO Panel appreciated this concise summary on the 90-day rat feeding study, and the conclusion reached by France. The characteristics of the maize grains of test and control maize used for dietary preparation and the resulting diets are provided in the appendices B-E on pages 451-483 of the report on the 90-day rat feeding study (WI-2007-064 2008). WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			It would have been desirable to clarify the cultivation conditions (possible exposure to drought stress) under which the maize evaluated in the subchronic toxicity study was raised.	
France	D, 07.08 Toxicology	Ministère de l'Economie (Consommation)	Protein NptII is expressed in a number of transgenic plants as a selection marker (maize MON863, cotton MON1445 and MON15985, Amflora potatoes). The factors that allow the safety of the protein to be demonstrated have already been submitted to and evaluated by the French Food Safety Agency [AFSSA] (*3) and the European Food Safety Authority [EFSA] in the marketing applications for these plants. The rapid breakdown in vitro of protein NptII in simulated gastric and intestinal fluid (cf 7.9 Allergenicity). The safety of protein NPTII has, moreover, been the subject of detailed reports by the evaluation agencies: AFSSA concluded in 2002 that 'the consumption by humans or animals of food products consisting of or produced by genetically modified plants containing genes for resistance to kanamycin and/or ampicillin presents only a theoretical – and in any case negligible – risk to human and animal health'. More recently, EFSA concluded in its March 2009 report that an adverse effect on health due to the consumption of genetically modified plants containing genes for resistance to kanamycin and/or ampicillin is unlikely. However, in genetically modified plants intended for consumption by humans or animals AFSSA recommends the avoidance of genes for antibiotic resistance likely to have deleterious effects on human and animal health. (*2) The equivalence between the protein NptII expressed in maize MON87460 and that produced by E coli has been demonstrated as for CspB. (*3) http://www.afssa.fr/Documents/BIOT-Ra-ConsoOGM.pdf	The EFSA GMO Panel considers that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants. Section 5.2. of the opinion summarizes the findings on the potential toxicity of the CspB protein, including a consideration of the safety of the source organism (including a history of presence in the food chain, e.g. in natto and its "qualified presumption of safety" as a producer micro-organism), as well as the outcomes of the in-vitro, in-silico, and in-vivo experiments

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	General comments	Federal Agency for Nature Conservation (BfN)	The Federal Agency for Nature Conservation considers that further information is required before the risk assessment of EFSA/GMO/NL/2009/70 can be finalised (see specific comments). Stress tolerance, as in MON87460, is of high environmental concern. Despite the restricted use of the GMO for import and processing it is very likely that viable plant material (e.g. grain) will enter the environment via loss and spillage. MON87460 expresses the prokaryotic CspB protein under control of the constitutive Ract1 promoter. Cold-shock proteins (CSP) naturally occur in pro- and eucaryotes and are implicated to be involved in stress-tolerance. To our understanding, the mode of action of CspB is very unspecific as the protein influences the expression of proteins by unspecifically binding single-stranded nucleic acids and destabilizing their secondary structure. The applicant provides data that demonstrates the unspecific RNA-binding ability of plant-expressed CspB, the ability of CspB to destabilize secondary structures, the cytoplasmic and nucleic localization of the protein in MON87460 and the accumulation of the protein in rapidly growing tissue and developing reproductive organs ([redacted]). However, no details on the actual mechanism by which CspB enhances drought-tolerance is given. The information on the mode of action of CspB is therefore insufficient and the applicant is asked to provide and test hypotheses how the expression of the new CspB protein relates to the intended trait and other possible stress reactions. Considering the unspecific RNA-binding ability of CspB and the constitutive expression of the protein, the probability of CspB influencing the expression of a wide variety of plant proteins must be considered as high. This could lead to extensive unintended effects that should be carefully investigated for example by comprehensive transcriptomic and proteomic analyses.	The agronomic characteristics of maize MON 87460 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns. There are no data on cold tolerance as such. However, the data on survival shows none or declining populations under feral conditions, indicating that if there is cold tolerance it is not affecting survival characters.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			In the dossier the information to assess the mechanism of action as well as the phenotypic characteristics (both agricultural and ecological) need to be expanded. Additional information is necessary to decide whether i) tolerance to other stressors than drought were conferred and ii) to decide whether accidentally spilled MON87460 grain during transport and processing may persist and potentially establish in the different environments in Europe. For most studies replication and sample size is weak. and a statistical power analysis needs to be carried out for each study to determine their quality. With regard to the environmental risk assessment, studies on the occurrence of volunteers and on the survival of the GMO in unmanaged environments are of special importance. The information submitted so far does not allow to conclude on these issues.	
Germany	General comments	Federal Agency for Nature Conservation (BfN)	A further major concern is connected to the presence of the antibiotic marker gene nptII in MON87460. The gene not only provides a resistance for kanamycin but also for neomycin, geneticin (G418), gentamicin A/B, paromomycin, and framycetin. Kanamycin and neomycin are categorised as highly important antimicrobials by WHO (2007) and EMEA (2007). The EFSAGMO-panel (2009) identified limitations related among others to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source. In addition the spatio-temporal relationship between the prevalence of antibiotic resistance and selection pressure is judged not to be fully understood. Therefore we are of the opinion that because of the above mentioned uncertainties in combination with the high amount of antibiotic marker genes present in the GM-plant tissue and the importance of the corresponding antibiotics adverse effects to human and animal health can not be excluded and the precautionary principle should prevail.	1/ Having considered the information provided in the technical dossier and the additional information provided by the applicant on 04/10/2010 and 30/04/2012 upon request of the EFSA GMO Panel, as well as relevant publications published in the scientific literature, the EFSA GMO Panel concluded that adverse effects on human and animal health and the environment resulting from the transfer of the nptII and cspB genes present in maize MON 87460 to bacteria are unlikely, because of a highly limited potential for gene transfer. Taking into account the different exposure routes, this conclusion is mainly based on the following assessment: (1) the integration of the nptII and cspB genes through non-homologous recombination is most unlikely; (2) enhanced horizontal transfer of the nptII gene due to Cre-lox mediated recombination is unlikely; (3) the stabilisation of the nptII gene into bacterial cells by double

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			Furthermore the use of antibiotic marker genes, especially in new constructs, is not state-of-the-art and in contradiction to the intentions of Directive 2001/18/EC, which foresees a phasing-out of AR-genes. In fact genetic information (loxP) has been integrated in MON87460 which should allow marker gene removal. It is yet unclear why the nptII gene has thus not been removed.	homologous recombination of A. tumefaciens sequences flanking the nptII gene, and subsequent dissemination in the environment are unlikely; and (4) the unlikely but theoretically possible transfer of the nptII and cspB genes in maize MON 87460 to bacteria via gene replacement does not raise concerns due to the lack of an additional selective advantage which would be provided to the recipients in the receiving environments. The probability of horizontal gene transfer of the insert DNA of maize MON 87460 remains several orders of magnitude lower than the gene transfer efficiencies between bacteria. Therefore, its contribution (if any) to the environmental prevalence of nptII genes is negligible. In summary, the analysis of horizontal gene transfer from maize MON 87460 to bacteria did not indicate a risk to human or animal health or to the environment in the context of its intended uses.
Germany	General comments	Federal Agency for Nature Conservation (BfN)	2/ As far as reported or traceable, several studies used test, control or reference material contaminated with MON87460 or maize NK603 (Sammons et al. 2008 MSL-21353; Rosenbaum and Eberle 2008 MSL-21426; Whitsel 2007 MSL-20779; Eberle 2009 MSL-21857; [redacted] MSL-21408; [redacted] WIL-50342) which does not comply with good laboratory practice standards and impedes the interpretation of the study results. 3/ The applicant's proposal for an environmental monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC). As stress tolerance may be directly related to the plant's fitness a strict environmental monitoring is crucial.	2/ The EFSA GMO Panel also noticed that the conventional counterpart and non-GM maize commercial reference varieties seed produced and sown for some of the experimental studies contained event MON 87460 up to a level of 1.84%, and maize MON 87460 seed sown for some experimental studies contained the event NK603 at a level of ≤1.84%. The levels of adventitious presence of MON 87460 and NK603 were low and were deemed by the EFSA GMO Panel to have no negative effect on the quality of the studies or interpretation of the results. 3/ The EFSA GMO Panel considered that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. As the scope of the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			EFSA, European Food Safety Authority, 2009, Statement of EFSA on the consolidated presentation of opinions on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal (2009) 1108, 1-8 EMEA, Committee for Medicinal Products for Veterinary Use and Committee for Medicinal Products for Human Use, 2007, Presence of the Antibiotic Resistance Marker Gene nptII in GM Plants for Food and Feed Uses, EMEA/CVMP/56937/2007 World Health Organisation, 2007, Critically Important Antimicrobials for Human Medicine: Categorization for the Development of Risk Management Strategies to contain Antimicrobial Resistance due to Non-Human Antimicrobial Use, Report of the Second WHO Expert Meeting Copenhagen, 29–31 May 2007	application EFSA-GMO-NL-2009-70 does not include cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable grains of maize MON 87460 during transport and processing for food and feed uses, and with the exposure through manure and faeces from animals fed maize MON 87460 grains. The environmental risk assessment identified no potential adverse effects to the environment. The EFSA GMO Panel considered that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of conventional maize varieties. Therefore, the EFSA GMO Panel concluded that no case-specific monitoring is necessary.
Germany	D, 02 Information on the sequences actually inserted or deleted	Federal Agency for Nature Conservation (BfN)	The data submitted for molecular characterisation of GM maize MON87460 consist of Southern blots, PCRs and sequence information, demonstrating the presence of a single insertion of a single copy of the T-DNA containing the cspB and nptII expression cassettes (Skipwith et al. 2007). 1/ The insertion retained the sequence and organisation of the vector but was truncated. 733 bp of the RactI promoter and leader region were deleted. The applicant is asked to provide data showing how the intended promoter function is influenced by this severe truncation. 2/ Furthermore, it is not clear, why the applicant refrained from the removal of the nptII expression cassette from the GMO, especially if one considers that the necessary loxP sequences were introduced into the GMO.	1/ Information concerning the activity of the truncated RactI promoter has been requested by the EFSA GMO Panel and provided by the applicant (additional information 04/10/2010). 2/ The EFSA GMO Panel requested further information concerning the presence of the *nptII* gene and the possibility for horizontal gene transfer. Please see the scientific opinion and additional information provided on 04/10/2010 and 30/04/2012 for further details.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D, 03 Information on the expression of the insert	Federal Agency for Nature Conservation (BfN)	Expression of the CspB and NptII proteins was assessed in various tissues (some at different growth stages) of MON87460 maize through ELISA. Samples were collected during a field study at six individual sites in the U.S. over one season (2006) (Mozaffar & Silvanovich, 2008; MSL-21033) and at four individual sites in Chile over one season (2006-2007) (Shi et al., 2008; MSL-21731). At each site, three replicated plots of the test line and a conventional maize line with a similar genetic background to the test plants were planted using a randomized complete block design. Only at the Chilean sites plots were divided into subplots with well-watered or water-limited conditions. Only descriptive statistics (mean, standard deviation, min/max levels) were calculated across sites. Significant differences between the single locations were not evaluated. The trial sites were insufficiently described. The applicant is asked to provide the cited production plans (Mulesky 2007, MSL-20810; Adu-Tutu 2008, MSL-21759). Since protein expression in plants can be affected by climatic conditions, soil fertility, agricultural practice or unknown gene-environment interactions, data from a single season give a rough estimate of expression levels only. A more robust and reliable data basis should, therefore, include data from at least three field seasons at the same location (with six locations representing different environmental conditions) to integrate possible differences in expression values triggered by differences in ecological conditions. Criteria on which the representativeness of locations has been established should be given, and the environmental conditions should be documented and provided with the application. Statistical analyses should include differences between sites and locations. The applicant is asked to statistically test the hypothesis that expression of CspB is not triggered by water stress. Furthermore, the	The agronomic characteristics of maize MON 87460 together with the compositional analysis did not indicate the occurrence of unintended effects that would cause safety concerns. The aim of the field trials for protein expression experiments is to gather information on the ranges of protein expression levels. The use of different sites exposes the plants to a range of environmental conditions. Therefore, these trials are considered sufficient by the EFSA GMO Panel. The toxicity of the CspB has been analysed. The compositional analyses were also performed under water limited and normal irrigation conditions and no biological significant differences were observed. The following production plans, among others, were provided as additional information received on 4 October 2010: Adu-Tutu, K., et al. (2008) Amended Report for MSL0021095: Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460, MON 87460 × NK603, MON 87460 × MON 89034 × NK603, and MON 87460 × MON 89034 × MON 88017 in Chile during 2006-2007. Production Plan #: 06-45-B3-02. Amended 1 - MSL0021759. Monsanto Co., St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012] Mulesky, M., et al. (2007) Field Production of Tissues and Grain from Drought Tolerant Corn MON 87460 in the U.S.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			examination of the expression of CspB under other stress conditions (heat, cold, etc.) has not been performed but is essential considering the unspecific mode of action of CspB and the involvement of the protein in general stress-tolerance (see also D.4).	during 2006. Production Plan: 06-01-B3-04. MSL-0020810. Monsanto, St. Louis, USA [part of confidential dossier information, additional information dated 4 October 2012]
Germany	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Agency for Nature Conservation (BfN)	The applicant submitted several studies to gain information on possible alterations of MON87460 maize regarding reproduction, dissemination, and survivability. After the assessment of these studies the BfN comes to the conclusion that further information (amended/additional studies) are necessary to decide whether i) tolerance to other stressors than drought were conferred and ii) to decide whether accidentally spilled MON87460 seed during transport and processing may persist and potentially establish in the different environments in Europe. For most studies replication and sample size is weak and a statistical power analysis needs to be carried out for each study to determine the quality of the studies. Weak data were also provided for improved yield and phenotypic improvements of MON87460 maize under water stress. With regard to the environmental risk assessment studies on a possible extension of the cultivation area, the occurrence of volunteers and on the survival of the GMO in unmanaged environments are of special importance. The information submitted so far does not allow to conclude on these issues. Volunteers: Experiments for changes in volunteer occurrence focus on the study of Whitsel (2008; MSL-21008). Using small plots and data from a single year, Whitsel (2008; MSL-21008) found no volunteer maize plants either from MON87460, the control line, or any other reference line. However, this study does not sufficiently explore on possible changes in the biology of MON87460 with regard to the occurrence of volunteers. Much larger scale experiments with standard agricultural practice in different climates are necessary to	The EFSA GMO Panel refers to the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. In the present case, the EFSA GMO Panel considered the persistence and invasiveness studies performed in the USA sufficient. Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable grains from maize MON 87460 during transport and processing. The EFSA GMO Panel considered it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			assess possible changes. The experimental sites selected by the applicant all faced severe frost (between -20 and -10 °C) and hence do not represent the geographic and climatic environments for which an impact of MON87460 spillage in the EU should be assessed. Maize volunteers (up to 10%) occur frequently in southern parts of Europe (Palaudelmàs et al. 2009) where frost is less severe. Experiments in Spain (Palaudelmàs et al. 2009) were carried out over three years (2004-6) in 12 fields. Volunteers were recorded in all fields in densities between 34 and 8730 volunteers/ha. Thus volunteers frequently occur in southern parts of Europe where water stress is common. The applicant is requested to submit additional studies to answer the question whether the stress tolerance induced in MON87460 will affect the weed potential (e.g. number of volunteers). Thus, volunteers should have been monitored in the agronomic/ecological studies presented by the applicant (e.g. MSL-21120; MSL-21353; MSL-21857; MSL-22393)	or isolated grains shed in the field during harvesting, indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g., Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Survival of maize plants outside cultivation in Europe is mainly limited by: a combination of low competitiveness; absence of a dormancy phase; and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MON 87460, drought tolerance alone is not likely to provide a selective advantage outside cultivation in Europe. Therefore, the EFSA GMO Panel considered it very unlikely that maize MON 87460 will differ from conventional maize varieties in their ability to survive until subsequent seasons, or to establish feral populations under European environmental conditions. Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17. Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Agency for Nature Conservation (BfN)	Survival: Rosenbaum & Eberle (2008; MSL-0021462) tested the survival of MON87460 in unmanaged habitats. The experiments were carried out during a single vegetation period at four locations. The representativeness of locations compared to the receiving environments in Europe has not been established. Moreover, the study has several further shortcomings. The sample size of seeds used was very small, especially when it is considered that only a fraction of the used F2 hybrids will be stress tolerant. To our understanding the study thus cannot create sufficient data for the ERA. We strongly recommend to repeat/modify the study using a substantially increased sample and plot size. Despite the mentioned deficits the study indicates that MON87460 maize will survive and reproduce in some habitats. This is in accordance with Palaudelmàs et al. (2009), who not only assessed the occurrence of maize volunteers in Spain but also provided data on the survival and reproduction of maize. In accordance with Rosenbaum & Eberle (2008) it must be assumed that volunteer (or spilled) maize can reproduce regularly under certain climatic conditions e.g. present in some regions of Europe. Because water stress must be considered to act as a strong selection pressure and the loss and spillage of MON87460 maize occurs certainly when commercially used, additional studies are necessary to assess changes of MON87460 maize with regard to reproduction, dissemination and survival. Dormancy: Whitsel (2007; MSL-20779) experiments on dormancy and germination of MON87460 maize did not indicate differences for the studied parameters. However, the study cannot be regarded as fully sufficient for the environmental risk assessment. In general the influence of additional abiotic stressors (including e.g. water stress, cold, and freezing) should be included in the experimental setup to test for differences in dormancy. In Whitsel (2007) experiments were	The EFSA GMO Panel refers to the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. Considering the intended uses of maize MON 87460 and the physical characteristics of maize seeds, possible pathways of gene dispersal are (accidental) grain spillage during transport and processing and the dispersal of pollen from occasional feral GM maize plants originating from grain spillage. The EFSA GMO Panel considered it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions (mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting, indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g., Gruber et al., 2008).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			carried out with temperatures > 5°C. Thus temperature below zero, which will be characteristic for many European regions, have not been assessed. The applicant is requested to amend the study, including the influence of water stress and frost-hardiness on the germination and possible dormancy of seed. In addition the statistical power of the study should be calculated and documented. To assess the influence of the newly CspB protein in relation to dormancy and abiotic stressors, we recommend recording expression levels of CspB during germination for each treatment. Because loss and spillage will be the main exposure path which has to be considered for application EFSA-GMO-NL-2009-70 experiments should be carried out with both F1 and F2 seed. Pollen viability: A study on the viability of MON87460 pollen has been submitted (Whitsel & Sammons 2008 - MSL-21232). The applicant is asked to clarify in which way and under which conditions water stress will influence pollen and pollen viability. In the results presented water stress did influence neither MON87460 nor the control or reference lines. Pollen viability over time, which is crucial for vertical gene flow has not been assessed.	However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Even though GM maize plants outside cropped area have been reported in Korea, as a result of grain spillage during import, transport, storage, handling and processing (Kim et al., 2006; Lee et al., 2009; Park et al., 2010), survival of maize plants outside cultivation in Europe is mainly limited by: a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens, herbivores and frost. Since these general characteristics are unchanged in maize MON 87460, drought tolerance is not likely to provide selective advantages outside cultivation in Europe. Therefore, as for any other maize varieties, GM maize plants would only survive in subsequent seasons in warmer regions of Europe and are not likely to establish feral populations under European environmental conditions. The extent of cross-pollination to other maize varieties will mainly depend on the scale of accidental release during transport and processing, and on successful establishment and subsequent flowering of this GM maize plant. For maize, any vertical gene transfer is limited to other *Zea mays* plants as populations of sexually compatible wild relatives of maize are not known in Europe (Eastham and Sweet, 2002; OECD, 2003). The flowering of occasional feral GM maize plants originating from accidental release occurring during transport and processing is unlikely to disperse significant amounts of GM maize pollen to other maize plants. Field observations performed on maize volunteers after GM maize

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
				cultivation in Spain revealed that maize volunteers had a low vigour, rarely had cobs and produced pollen that cross-pollinated neighbour plants only at low levels (Palaudelmàs et al., 2009). Eastham K and Sweet J 2002. Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. European Environment Agency. Available from http://www.eea.europa.eu/publications/environmental_issue_report_2002_28 Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17. Kim C-G, Yi H, Park S, Yeon JE, Kim DY, Kim DI, Lee K-H, Lee TC, Paek IS, Yoon WK, Jeong S-C and Kim HM, 2006. Monitoring the occurrence of genetically modified soybean and maize around cultivated fields and at a grain receiving port in Korea. Journal of Plant Biology, 49, 218-223. Lee B, Kim C-G, Park J-Y, Park KW, Kim H-J, Yi H, Jeong S-C, Yoon WK and Kim HM, 2009. Monitoring the occurrence of genetically modified soybean and maize in cultivated fields and along the transportation routes of the Incheon Port in South Korea. Food Control, 20, 250-254. OECD 2003. Consensus document on the biology of Zea mays subsp. mays (Maize). Series on Harmonisation of Regulatory Oversight in Biotechnology (ENV/JM/MONO(2003)11), 27, 1-49. Available from http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT00004

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
				26E/$FILE/JT00147699.PDF Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594. Park KW, Lee B, Kim C-G, Kim DY, Park J-Y, Ko E-M, Jeong S-C, Choi K-H, Yoon WK and Kim HM, 2010. Monitoring the occurrence of genetically modified maize at a grain receiving port and along transportation routes in the Republic of Korea. Food Control, 21, 456-461.
Germany	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Agency for Nature Conservation (BfN)	Resistance to abiotic stressors including water stress: The resistance of MON8740 to abiotic stressors was tested in several greenhouse and field experiments. Greenhouse studies were carried out to test for different reactions of MON8740maize due to water stress (Eberle et al. 2009 - MSL-22343; Chomet et al. 2008 - MSL-21719), salt stress (Whitsel 2008 – MSL-21615), heat stress (Eberle et al. 2008 – MSL21593) and cold stress (Eberle et al. 2008 – MSL-21509). For all studies the link between the stressor and the levels of CspB has not been monitored leaving the information on the molecular response of MON87460 incomplete. All of the above studies lack a statistical power analysis. Because sample sizes were often small a power analysis should be provided in retrospect. The information on the tolerance to water stress of MON87460 should be amended. MON87460 failed to show statistically significant yield increase (Luthey 2009, MSL-22168) or increased phenotypic performance (Eberle et al. 2009 – MSL-22343). With regard to Chomet et al. 2008 (MSL-21719) the applicant is asked to identify the control maize line and to provide additional quantitative data on the exercised water stress. Whitsel et al. (2008; MSL-21615) tested the reaction of MON87460 to	The EFSA GMO Panel concluded that depending on the treatment, differences were observed between maize MON 87460 and its conventional counterpart. Given the intended trait, the observed differences are not unexpected, and indicate no safety concern. Further, the EFSA GMO Panel considers that these data should be put into the context of the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. Considering the intended uses of maize MON 87460 and the physical characteristics of maize seeds, possible pathways of gene dispersal are (accidental) grain spillage during transport and processing and the dispersal of pollen from occasional feral GM maize plants originating from grain spillage. Overall, the EFSA GMO Panel considered that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			salt stress in a greenhouse study. MON87460 showed a significant increase in chlorophyll content and plant vigor during moderate salt treatment. These results should not be dismissed as done by the applicant but trigger additional studies which differentiate salt levels in a more graduate way. Given the fact that plants gradually adapt to increasing salt levels multi-generation studies and/or studies in which seedlings already germinate in salted soils should be carried out. The assessment of heat stress (Eberle et al. 2008 – MSL-21593) should have been combined with water stress as both factors may be correlated in the field. The provided field tests of MON87460 with regard to abiotic stress must be criticized as the information provided is insufficient (see D.7.4). Palaudelmàs,M., Pefias,G., Melé,E., Serra,J., Salvia,J., Pla,M., Nadal,A. & Messeguer,J. (2009) Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.	conventional maize varieties.
Germany	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	Federal Agency for Nature Conservation (BfN)	Concerning the data on segregation (Rosenbaum, 2008 – 07-RA-B3-01), it is not clear how the backcross generations were gained and how their relation to the R0 generation is. The provided breeding history describes only the generations used for Southern blot analyses. The applicant is asked to provide this information.	The EFSA GMO Panel considered the data provided sufficient to conclude on the stability of the insert. A description of the generations used for the segregation analyses was provided (Rosenbaum, 2008). Rosenbaum, E.W. (2008) Assessment of insert segregation for MON 87460. Report number 07-RA-B3-01. Monsanto Co., St. Louis, USA [as part of confidential dossier information]
Germany	D, 07.01 Comparative assessment	Federal Agency for	Alba et al (2008; MSL-21752) did not discuss the reason for the high levels of abscisic acid in two of the three replicates for forage grown under well watered conditions at site Colina. In our opinion the 4.5 to 7.5 times higher level could indicate a site specific accumulation of the component, which is generally associated with stress responses,	It is the understanding of the Panel that all replicates for forage grown at site Colina both under well water conditions and under water-limited conditions were included in the statistical analysis reported in [Alba et al, 2008]: "*The raw data shows that these statistically significant differences*

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Nature Conservation (BfN)	in the GMO. This can be regarded as an unexpected effect and must be further observed. The applicant is requested to assess, whether the levels of abscisic acid relate to the environmental conditions present at the site in question. At present site effect (gene-environment interaction) can neither be denied nor proven. Therefore with regard to a final assessment, further information is required, because the information provided is not considered sufficient to support the conclusion of a substantial equivalence of MON87460 maize to conventional maize, which is the basis of further conclusions in the application.	*originate from two replicates of MON 87460 at the CL site. The forage tissue from these two replicates had levels of abscisic acid (85.2 ppb, 122 ppb) that were 4.5 to 7.5 times greater than the overall mean of this metabolite in forage. Forage from the third replicate from the CL site contained 18.5 ppb abscisic acid, which is nearly identical to the overall mean of this metabolite in forage from the CL, CT, and LUM sites. Thus, the levels of abscisic acid in forage at the CL site were extremely variable.*" PRESS residuals were used to identify outliers. Only the abscisic acid value in MON 87460 forage obtained from site Calera de Tango (CT) was considered a true outlier (PRESS Std Residual > 7) and removed from further analysis [Alba et al, 2008].
Germany	D, 07.04 Agronomic traits	Federal Agency for Nature Conservation (BfN)	MON87460 maize has been engineered to differ in agronomic aspects in comparison to conventional maize. It is therefore noteworthy that the applicant could not demonstrate a statistically significant rise in MON87460 yield under water stress (Luethy 2009, MSL-22168). This may connect to shortcomings in the study design which can be found in many other studies (see below) and compromise the ERA. Apart from the study of Luethy (2009) other experiments regarding the agronomic and phenotypic characteristics of MON87460 maize in comparison to conventional maize were carried out in the USA and in Chile over several years and multiple locations. Not all experiments included water-limited conditions. The latter were included as a treatment in 2006-2007 in Chile (four sites) and 2007 in the USA (three sites). The submitted data do not allow to conclude on the agronomic and phenotypic equivalence of MON87460 maize. Further information, including additional replicates/sites/years and additional analyses are	Maize MON 87460 is designed to maintain a higher yield than comparators under similar stress conditions – not to increase yield when stressed. There is no indication that other characteristics are significantly higher than comparators under similar stress levels – in fact the opposite appears to be the case (e.g., reduced vegetative growth in order to protect the yield of grain).

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			judged necessary for the ERA. The applicant is asked to clearly analyse the expected – and potential unexpected – characteristics of MON87460 maize. Shortcomings of the presented studies can be summarized as follows: I. Although the agronomic characteristics addressed do not indicate a potential for differences in reproduction, dissemination and survivability of MON87460 maize, the selected parameters themselves cannot sufficiently indicate such changes. II. The representativeness of the chosen locations and management practices has not been established. For the ERA representativeness should be demonstrated for the environments where the cultivation of MON87460 takes place and, to some extend, for the receiving environments in Europe, where loss and spillage of MON87460 are the main concern. III. Some of the characteristics measured were based on very small sample sizes of five plants per plot only. A statistical power analysis should be carried out to assist the assessment of the validity of the tests. IV. Within the individual-site analyses showing significant differences between test and control were mostly not compared to any reference data. V. Within the combined-site analyses showing significant differences between test and control were compared to the minimum and maximum of all references, even though the same references are not grown at all sites	
Germany	D, 07.04 Agronomic traits	Federal Agency	VI. The control and the references are different for the different studies (years and sites). It is not possible to compare the results of the different studies and years. VII. Differences between test and control in the individual-site	Depending on the parameter used, quantitative or semi-quantitative measures were reported. Water stress was established based on an indicator for soil water availability. Many studies on agronomic and phenotypic characteristics

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		for Nature Conservation (BfN)	analyses should be assessed relative to references from the respective sites only. A summarising statistical analysis should address the between-site variation of the data. VIII. Statistical analyses of gene-environment interactions are missing and should be provided. IX. Data presented on disease incidence and insect damage are of limited value because maintenance chemicals were applied throughout the whole growing season and the information of the practices of the application of these chemicals are missing. X. Rated data for abiotic and disease stress and information on prevailing pest and disease pressure (baseline) are missing and required to assess ecological interactions and to estimate whether the data collected during the test season are representative and typical for the respective sites. XI. The field design of experiments to obtain data on disease incidence and insect damage cannot considered to be suitable because of the small plot size (3 m x 6 m to maximum 6m x 9 m). The chosen field design thus is not comparable to common agricultural practice and not typical for field studies with a focus on biological interactions (e.g. arthropod abundance).	were provided by the applicant. From the data provided it could be concluded that, under certain conditions and in certain locations maize MON 87460, was different from its conventional counterpart. Because these differences were small and within background ranges, they did not raise safety concerns (summarized and explained in more detail in the Scientific Opinion).
Germany	D, 07.08 Toxicology	Federal Agency for Nature Conservation	D.7.8.1. Safety assessment of newly expressed proteins In the acute toxicity study of Cold Shock Protein B (CRO-2007-182, 2008) only a single oral dose of 4.70 mg CspB/kg body weight was administered to mice. No adverse effects were observed at this dose. However, according to OECD Test Guideline 401 at least three dose levels should be tested. When performing a limit test (with one dose level) at least 2000 mg/kg bodyweight should be tested (OECD, 1987). In addition acute toxicity testing of newly expressed proteins is of little value for the risk assessment of repeated human and animal consumption of GM food/feed (EFSA, 2008). Hence, the	As explained in the opinion, the conclusion on the safety of the newly expressed CspB protein is based on the totality of data among others on the function and characteristics of the CspB protein, the qualified presumption of safety and natural occurrence in the food chain of its source organism, the lack of similarity to known toxins, and its rapid in-vitro degradation by proteases. In other in-vivo experiments with the whole product, no effects were observed either. To request an additional pepsin-resistance test with RNA-bound CspB is considered unlikely to add much to the data

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		(BfN)	applicant is requested to perform a repeated dose 28-day oral toxicity study in rodents according to OECD Test Guideline 407 (OECD, 1995) with the newly expressed CspB protein. The results of the in vitro digestibility study (Kapadia et al. 2008) indicate that Escherichia coli-produced CspB protein is readily digestible in in vitro systems. However, because the CspB protein expressed in corn can be adsorbed to organic food components and is stabilized by binding to RNA molecules, its in vivo digestibility may be different. Therefore, it is recommended to investigate the in vivo digestibility of CspB protein in MON87460 maize. EFSA (2008) Updated Guidance Document for Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The EFSA Journal. 727, 1-135 OECD (1987) OECD Guideline for Testing of Chemicals. Test No. 401: Acute oral toxicity (adopted 24 February 1987). 1-7 OECD (1995) OECD Guideline for Testing of Chemicals. Test No. 407: Repeated Does 28-day Oral Toxicity Study in Rodents (adopted 27 July 1995). 1-8	already provided Under the conditions of pepsin resistance tests (low pH), the RNA-protein binding is likely to be implicated by RNA protonation (positive charge). In addition, RNA will be prone to certain chemical reactions at pH<2 and therefore less stable. With regard to RNA stabilization at higher pH values, Schindler et al. (1999) studied trypsin-mediated degradation of CspB and CspB bound to RNA at physiological pH values (http://hwmaint.jbc.org/cgi/content/full/274/6/3407). While the CspB bound to RNA was less rapidly degraded than CspB alone, the results from the study by Schindler et al. show that under influence of trypsin, more than 80% of the RNA-bound CspB is still degraded at pH 7 after 120 minutes. Trypsin digestion is not the primary test of the degradation assays recommended by guidance (e.g., EFSA, Codex). Schindler, T., et al. (1999) The family of cold shock proteins of Bacillus subtilis. J. Biol. Chem, 274(6): 3407-3413. Available online at: http://hwmaint.jbc.org/cgi/content/full/274/6/3407

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D, 07.08 Toxicology	Federal Agency for Nature Conservation (BfN)	D.7.8.4. Toxicological testing of the whole GM food/feed In the 90-day feeding study with rats ([redacted]- WIL-50342) neither the homogeneity and stability nor the concentrations of the ground test or control substance in the formulated diets were determined. As far as traceable the test substance MON87460 used in this study was contaminated with NK603 maize (for substance identity compare Lot no. in [redacted]. and Eberle 2008 MSL0021857). Also, although the composition of the test diets were analysed, details about the origin of their components - apart from the test and control substances - are missing. Therefore it cannot be verified whether further contaminations with GMO exist. The administration of grain from MON87460 to rats for at least 90 consecutive days at concentrations up to 33% (w/w) in the diet led to significant differences in some parameters of serum chemistry (around 30% increased alkaline phosphatase level in the females in the 11% MON87460 corn group, around 29% decreased aspartate aminotransferase level in females in the 33% MON87460 corn group). In addition, some significant differences in organ weights were observed (around 9% lower heart weight: absolute and relative to brain weight in males fed 33% test substance MON87460, 14 % lower thyroid weight relative to body weight in females fed a diet with 33% MON87460 group). Although WIL Research Laboratory claims that all of the observed significant differences fall within historical control range values of the laboratory, these effects should not be neglected. In fact, the effects may evolve stronger when animals or humans are exposed over longer periods or even chronically. Hence it is recommended to perform a chronic toxicity study in mammals preferable including reproduction endpoints. In the 42-day broiler study ([redacted]) the body weight of chickens	This kind of laboratory animal feed can stay stable for at least six month while the experiment had been carried out within a shorter timeframe (as described in previous dossiers, based on information obtained from laboratory animal feed producers). Appendix B (page 456) of WI-2007-064 (2008) shows that PCR tests on maize MON 87460 tested negative for NK603. The differences in various parameters in the 90-day trials are assessed in the pertinent part of the EFSA GMO Panel Scientific Opinion, section 5.1.3, as follows: "All animals survived the treatment period and there were no relevant clinical signs. Body weights and feed consumption were comparable in all groups. Statistically significant differences that occurred only in the group fed 11% maize MON 87460, i.e., higher mean serum alkaline phosphatase activity and lower urine specific gravity in females, are not considered treatment-related due to the lack of a dose-response. A significantly lower aspartate aminotransferase activity in females fed diets containing 33% maize MON 87460 is not considered to be an indication of adverse effects. Mean sodium serum levels were slightly lower in females of the high-dose group but fell within the range of the historical control means. Males in the group fed a diet containing 33% maize MON 87460 showed a significantly lower heart weight, both in absolute terms and as relative ratio to brain weight but not in relation to body weight and females showed a lower thyroid and parathyroid weight in relation to body weight. The mean values fell within the range of the historical control means. There were no relevant findings in the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			was not determined for each individual but for groups of 20 chickens. Hence the parameter weight gain and feed consumption/weight gain were calculated on the basis of mean pen values. This procedure makes it more difficult to detect significant effects. The applicant should be advised to determine the individual chicken weight at study start in future. As with the feeding study in rats the test substance MON87460 was contaminated with NK603 maize. Also, starter and finisher diet contained about 30 percent soybean meal of unknown origin. Therefore it cannot be verified whether further contaminations with GMO exist.	histopathological examinations of these organs. Macroscopic and microscopic examinations of other selected organs and tissues did not reveal changes related to administration of the test materials. The EFSA GMO Panel concluded that there were no indications of adverse effects in this study." The EFSA GMO Panel agrees that analyses of data collected at the individual bird level would add value to the broiler feeding study. However, since the comparative safety assessment did not raise any nutritional concerns, and no other adverse effects were found, these analyses are not considered essential by the EFSA GMO Panel. WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D, 08 Post-market monitoring of GM food/feed	Federal Agency for Nature Conservation (BfN)	PMM of GM food/feed: The data provided to show the human and animal safety of MON87460 maize on the basis of its substantial equivalence to conventional maize (except for the introduced trait) are not sufficient. Therefore, a post-market monitoring for food and feed is recommended. The applicant is further requested to explain how the PMM of MON87460 maize in mixed GMO commodities imported, processed or used for food/feed will be realised. Because the monitoring of a GMO must be carried out on a case-by-case basis (Directive 2001/18/EC) with regard to species characteristics, modified traits, the intended use and the degree of exposition. Specific GM product quantities should be provided to estimate the degree of exposition. Otherwise, according to the precautionary principle, each imported and processed commodity must be assumed to contain any EU approved GM maize and consequently all parameters identified for the different GM maize products should then be monitored.	The risk assessment concluded that no data have emerged to indicate that maize MON 87460 is any less safe than its conventional counterpart. In addition, maize MON 87460 is as nutritious as commercial varieties. Therefore, and in line with the Guidance document (EFSA, 2011), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary. EFSA, 2011. EFSA Panel on Genetically Modified Organisms (GMO); Guidance for risk assessment of food and feed from genetically modified plants. EFSA Journal 9(5):2150.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D,12.1 General	Federal Agency for Nature Conservation (BfN)	The scope of this application is for import, processing, and all uses for food and feed. The applicant provides an environmental monitoring plan, which only covers adverse effects that may occur during handling and processing but fails to address areas such as effects resulting from loss and spillage of viable MON87460 maize and effects mediated via the characteristic trait of considerable environmental concern. The monitoring should serve as an early warning system. It 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). The monitoring plan fails to meet this goal but only presents a general idea about how the monitoring might be carried out. Thus, the monitoring plan does not meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC). It requires 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 EFSA GMO Panel considered that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. As the scope of the application EFSA-GMO-NL-2009-70 does not include cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable grains of maize MON 87460 during transport and processing for food and feed uses, and with the exposure through manure and faeces from animals fed maize MON 87460 grains. The environmental risk assessment identified no potential adverse effects to the environment. The EFSA GMO Panel considered that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of conventional maize varieties. Risks associated with a theoretically possible horizontal transfer from maize MON 87460 *npt*II and *csp*B genes to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns. Therefore, the EFSA GMO Panel concluded that no case-specific monitoring is necessary.
Germany	General comments	Federal Office of Consumer Prote	The scope of application EFSA-GMO-NL-2009-70 covers import and processing of maize MON 87460 including all feed and food products containing, consisting of, or produced from the genetically modified maize MON 87460. Cultivation is not covered by this application. The Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion that the data so far provided by the	The scope of the application includes import and processing for food and feed uses of maize MON 87460, and excludes cultivation. Considering the intended uses of maize MON 87460, the environmental risk assessment is concerned with the accidental release into the environment of viable grains from maize MON 87460 during transport and processing for food and feed uses, and with the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		ction and Food Safety	applicant are not sufficient to complete the evaluation of the application. Thus, further information is required to conclude on the risk assessment of dossier EFSA-GMO-NL-2009-70 (see specific comments). In addition, the provided monitoring plan is incomplete at this stage and needs further elaboration for implementation.	exposure through manure and faeces from animals fed maize MON 87460. In the case of accidental release into the environment of viable maize MON 87460 grains, there are no indications of an increased likelihood of establishment and spread of feral maize MON 87460 plants. Considering the intended uses of maize MON 87460 as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue due to the low levels of exposure. Due to its intended uses, exposure of bacteria in the environment, including those in the gastrointestinal tract, to recombinant DNA from maize MON 87460 is expected to be low. Risks associated with a theoretically possible horizontal transfer from maize MON 87460 *npt*II and *csp*B genes to bacteria have been analysed in detail, including different scenarios of integration, and did not raise safety concerns. In conclusion, the environmental risk assessment identified no potential adverse effects to the environment.
Germany	A, 07 Where appropriate, the conditions for placing on the market the food(s) or	Federal Office of Consumer Protection and Food Safety	The import documents should indicate that maize MON 87460 has not been approved for cultivation by the EC. Furthermore, appropriate measures have to be taken during transport, storage, and processing to avoid unintended release into the environment.	1/Not in the remit of the EFSA GMO Panel. 2/ The EFSA GMO Panel refers to the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in cases of accidental release of viable grains of maize MON 87460.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Germany	D, 02 Information on the sequences actually inserted or deleted	Federal Office of Consumer Protection and Food Safety	The applicant performed bioinformatics analyses of the MON 87460 flanking sequences to determine if any endogenous genes had been deleted and/or disrupted during the transformation event. However, a special bioinformatic analysis of the insert flanking regions to assess the presence and/or potential damage of genetic regulatory elements (such as potential promoter elements and polyadenylation sequences) is missing and should be asked for from the applicant. This particularly applies to a possible impairment of the putative promoter region belonging to the GenBank database sequence GI-226504777 which is located upstream of the 5' end of the insert and contains a coding sequence for a hypothetical protein (see Fig. 2 in Tu and Silvanovich, 2009). Tu, H. and Silvanovich, A. (2009) Updated bioinformatics evaluation of the DNA sequences flanking the insertion site in MON 87460: BLASTn and BLASTx analyses, Monsanto Technical Report, RAR-09-466, 1-30.☐	At this moment there are no reliable bioinformatic tools to detect promoter sequences. This information is mostly retrieved from the presence of annotated coding sequences which have been shown to be expressed. Both EST database and GenBank non-redundant nucleotide and amino acid databases were used to analyse the flanking sequences. The results indicated that it is unlikely that any gene is disrupted in the maize MON 87460. The agronomic and phenotypic characteristics of maize MON 87460 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns.
Germany	D, 04 Information on how the GM plant differs from the recipient plant in:	Federal Office of Consumer Protection and Food Safety	The applicant performed studies with maize MON 87460 at several sites in North and South America in order to evaluate volunteer potential and survival ability outside cultivation. It is concluded that no shifts in fitness or competitiveness were observed. Nevertheless, it remains unclear whether the tested environments represent potential receiving environments in Europe. Therefore, in order to adequately assess the effects of incidental spillage in the E.U., the applicant should be requested to comment on the transferability of the American data to European conditions. Because of the low likelihood of early detection, we recommend to elaborate on the risk assessment in this matter rather than to implement a case-specific monitoring.☐Figure 27 (technical dossier page 103) and Figure 28	1/ The EFSA GMO Panel refers to the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. In the present case, the EFSA GMO Panel considered the persistence and invasiveness studies performed in the USA sufficient. Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral maize plants in case of

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			(technical dossier page 105) are missing and, for the sake of completeness, should be complemented by the applicant.	accidental release into the environment of viable grains from maize MON 87460 during transport and processing. 2/ The missing figures were provided as spontaneous submission by the applicant (letter dated 03/10/2012). The EFSA GMO Panel refers to the scope of application EFSA-GMO-NL-2009-70. This application covers the import and processing of maize MON 87460 for food and feed uses, but excludes cultivation in the EU. Therefore, there is no requirement for scientific information of possible environmental effects associated with the cultivation of maize MON 87460. In the present case, the EFSA GMO Panel considered the persistence and invasiveness studies performed in the USA sufficient. Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that there are no indications of an increased likelihood of establishment and spread of feral maize plants in case of accidental release into the environment of viable grains from maize MON 87460 during transport and processing. The EFSA GMO Panel considered it very unlikely that the establishment, spread and survival of maize MON 87460 would be increased by the drought tolerance trait. Maize is highly domesticated and generally unable to survive in the environment without management intervention. Maize plants are not winter hardy in many regions of Europe; furthermore they have lost their ability to release seeds from the cob and they do not occur outside cultivated land or disturbed habitats in agricultural landscapes of Europe, despite cultivation for many years. In cultivation, maize volunteers may arise under some environmental conditions

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
				(mild winters). Observations made on cobs, cob fragments or isolated grains shed in the field during harvesting, indicate that grains may survive and overwinter in some regions, resulting in volunteers in subsequent crops. The occurrence of maize volunteers has been reported in Spain and other European regions (e.g., Gruber et al., 2008). However, maize volunteers have been shown to grow weakly and flower asynchronously with the maize crop (Palaudelmàs et al., 2009). Survival of maize plants outside cultivation in Europe is mainly limited by: a combination of low competitiveness; absence of a dormancy phase; and susceptibility to plant pathogens, herbivores and cold climatic conditions. Since these general characteristics are unchanged in maize MON 87460, drought tolerance alone is not likely to provide a selective advantage outside cultivation in Europe. Therefore, the EFSA GMO Panel considered it very unlikely that maize MON 87460 will differ from conventional maize varieties in their ability to survive until subsequent seasons, or to establish feral populations under European environmental conditions. Gruber S, Colbach N, Barbottin A and Pekrun C, 2008. Post-harvest gene escape and approaches for minimizing it. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 3, 1-17. Palaudelmàs M, Peñas G, Melé E, Serra J, Salvia J, Pla M, Nadal A and Messeguer J, 2009. Effect of volunteers on maize gene flow. Transgenic Research, 18, 583-594.
Germany	D, 07.02 Field trials	Federal	Production of material for comparative assessment:	The production plans for USA and Chilean trials (Mulesky, 2007; Adu-Tutu, 2008) were provided as part of the

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Office of Consumer Protection and Food Safety	With regard to the production of material for expression analyses as well as for comparative assessment the applicant refers to Monsanto Production Plan 06-01-B3-04 (Marcinkiewics, 2006 / Mulesky, 2007) and Monsanto Production Plan 06-45-B3-02 (Adu-Tutu, 2008). The applicant should be requested to provide these production plans in order to present details on the production of the used test and control material.	additional information supplied to EFSA in October 2010
Germany	D, 07.07 Anticipated intake/extent of use	Federal Office of Consumer Protection and Food Safety	Maize MON 87460 is to be used as any other maize in the E.U. including the production of foodstuffs. For this purpose, starch, maize syrups, ethanol, and maize oil are the essential commodities. The applicant estimates the anticipated intake based on food-balance-sheets. However, this method seems unsuitable for an exposure assessment within the risk assessment. The used approach depends on the regional sales volume for agricultural products and, in doing so, disregards that maize components are present in a lot of foodstuffs. Therefore, to carry out an exposure assessment within the population the amount of food eaten by the individual estimated from the national nutrition surveys should be counted back to its basic ingredients.	The GEMS data are widely used for estimates of food intake. As no nutritional issues have been identified, the exposure assessment has only limited applicability in this case. Exposure assessment based on trade data is a worst-case scenario assessment. The EFSA GMO Panel recognizes the value of estimating the human intake using individual food consumption data to assess dietary exposure at European level. For this reason, EFSA established an expert group, an EFSA network with representatives from each EU Member States (MS), to create the Comprehensive European Food Consumption Database, using 32 dietary surveys carried out in 22 MS. This database has recently been fine-tuned for dietary assessment for GM soybean. Tailoring this database for other GM crops is on-going.
Germany	D, 07.08 Toxicology	Federal Office of Consumer	D.7.8.1. Safety assessment of newly expressed proteins The applicant performed an acute toxicity study of CspB administered by the oral route to mice. The results of this study indicate that there were no adverse effects of CspB when administered to mice by single oral gavage at a dose of 4.7 mg/kg body weight (CRO-2007-182,	The EFSA GMO Panel considers that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Protection and Food Safety	2008). According to the applicant, this dose is three to four orders of magnitude above the conservative estimates for expected human exposures to CspB. However, we would like to point out that the administered dose of CspB is much smaller than recommended by the OECD (limit dose of 2000 mg/kg body weight) and does not correspond to common practice generally applied in comparable applications. By comparison, the NPTII protein, which was also evaluated within the scope of the present application, was administered to mice with a limit dose of 5000 mg/kg body weight (Fuchs et al., 1993). Thus, the applicant should be requested to explain in more detail the selected dose of 4.7 mg CspB protein per kg body weight as such a small-sized dose is neither recommended nor usual. Moreover, we point out that the acute toxicity study is unsuitable to calculate a MOE (margin of exposure) - as performed by the applicant - because MOEs are generally calculated based on long-term studies. CRO-2007-182. (2008) An acute toxicity study of Cold Shock Protein B administered by the oral (gavage) route to mice. Monsanto Technical Report, CRO-2007-182, 1-95. Fuchs, R. L., Ream, J. E., Hammond, B. G., Naylor, M. W., Leimgruber, R. M., and Berberich, S. A. (1993b) Safety assessment of the Neomycin Phosphotransferase II (NPTII) protein. Nature Biotechnology 11: 1543-1547. A detailed characterization of the NPTII protein produced in maize MON 87460 is missing (neither N-terminal sequence analysis nor	

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			MALDI-TOF MS nor analysis of biological activity are presented). Moreover, information about the identity of the E. coli produced NPTII protein and the NPTII protein expressed in maize MON 87460 is incomplete as only Western blot and SDS PAGE analyses are shown. However, neither a confirmation of equivalent functional activity nor glycosylation analyses are provided in order to demonstrate that the E. coli produced NPTII protein, used in the safety assessment of the protein (Fuchs et al., 1993b), was equivalent to the protein produced in maize MON 87460. The safety assessment of the NPTII protein presented by Fuchs et al. (1993b) refers to an E. coli produced NPTII protein that was shown to be chemically and functionally equivalent to the NPTII protein produced in genetically engineered cotton seed, potato tubers, and tomato fruit (Fuchs et al.,1993a). Therefore, the applicant should state if this equivalence verification is completely transferable on the NPTII protein expressed in maize MON 87460. Fuchs, R. L., Heeren, R. A., Gustafson, M. E., Rogan, G. J., Bartnicki, D. E., Leimgruber, R. M., Finn, R. F., Hershman, A., and Berberich, S. A. (1993a) Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. Biotechnology 11: 1537-1542. Fuchs, R. L., Ream, J. E., Hammond, B. G., Naylor, M. W., Leimgruber, R. M., and Berberich, S. A. (1993b) Safety assessment of the Neomycin Phosphotransferase II (NPTII) protein. Biotechnology 11: 1543-1547.□	
Germany	D, 07.08 Toxicology	Federal Offic	The results of a study of the acute toxicity of administration of the maximum dose of 4.7 mg/kg p.o. in the mouse show that the protein produced by E. coli equivalent (*1) to that of maize MON87460 does	The EFSA GMO Panel considers that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		e of Consumer Protection and Food Safety	not induce mortality after an observation period of 14 days. However, the dose employed is inadequate and incompatible with the objectives of such a study. A new study with a higher dose, in accordance with OECD guideline 420, needs to be submitted, particularly in view of the fact that protein CspB is new and has not previously been expressed in genetically modified plants intended for food use. (*1) The equivalence between the protein CspB expressed in maize MON87460 and that produced by E coli has been demonstrated: N-terminal sequence, MALDI-TOF analysis of peptides after trypsin digestion, molecular mass, absence of glycosylation, biological function.7.8) Evaluation of the safety of proteins CspB and NptII (continued). The safety of protein NptII is based on the following data: Protein NptII is ubiquitous in E. coli and therefore normally present in the human gastrointestinal tract.(· An in-silico study (FASTA) demonstrated the absence of sequence homology between protein NptII and proteins toxic to humans or animals listed in current databases. Protein NptII (*2) does not cause mortality in the mouse 7 days after administration of the maximum dose of 5000 mg/kg by the oral route.	consumption of food and feed derived from GM plants. Section 5.2. of the EFSA GMO Panel Scientific Opinion summarizes the findings on the potential toxicity of the CspB protein, including a consideration of the safety of the source organism (including a history of presence in the food chain, e.g. in natto and its "qualified presumption of safety" as a producer micro-organism), as well as the outcomes of the in-vitro, in-silico, and in-vivo experiments
Germany	D, 07.08 Toxicology	Federal Office of Consumer Protection	D.7.8.4. Toxicological testing of the whole GM food/feed With regard to the test and control maize material providing the basis for the 90-day toxicity study in rats as well as the broiler feeding study the applicant refers to Production Plan 06-45-B3-01 (Site Code: QUI, Chile). The applicant should be requested to provide this production plan in order to present details on the production of the used test and control material.□	The characteristics of the maize grains of test and control maize used for dietary preparation and the resulting diets are provided in the appendices B-E on pages 451-483 of the report on the 90-day rat feeding study (WI-2007-064 2008) WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		and Food Safety		Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]
Germany	D, 07.09 Allergenicity	Federal Office of Consumer Protection and Food Safety	1/ The provided bioinformatics analyses of sequence similarity of the CspB and NPTII proteins produced in maize MON 87460 to known allergens showed neither alignments to a stretch of eight contiguous amino acids nor alignments of at least 35% shared amino acid identity over stretches of 80 or more amino acids. Nevertheless, the applicant should be requested to provide information concerning the level of the greatest homologies found. 2/ Falsone et al. (2002) describe a protein from Cladosporium herbarum which was discovered as a minor allergen of this mould and which showed sequence homology to bacterial cold shock proteins (in the case of CspB from B. subtilis: 70% homology). In this respect, the applicant should be asked to comment on this data (Falsone et al., 2002) and to state whether this finding was considered within the assessment of allergenicity of the CspB protein (and - if not done by now - to make up for it). Falsone, S. F., Weichel, M., Crameri, R., Breitenbach, M., and Kungl, A. J. (2002) Unfolding and double-stranded DNA binding of the cold shock protein homologue Cla h 8 from Cladosporium herbarum. J. Biol. Chem. 277: 16512-16516.	1/ Based on experience, these most relevant alignments are not considered to be very informative if below the already stringent levels. 2/ The issue of the cold shock protein was raised and the applicant answered in 04/10/2010. It is considered that there is a low risk of the CspB protein being cross-reactive. This issue is also addressed in more detail in the allergenicity part of the EFSA GMO Panel Scientific Opinion.
Germany	D, 08 Post-market monitoring of GM food/feed	Federal Office of Cons	Since the risk assessment of maize MON 87460 cannot be finalised, it is not feasible to decide on the necessity of measures for post-market monitoring of GM food/feed.	The risk assessment concluded that no data have emerged to indicate that maize MON 87460 is any less safe than its conventional counterpart. In addition, maize MON 87460 is as nutritious as commercial varieties. Therefore, and in line with the Guidance document (EFSA, 2011), the EFSA GMO

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		umer Protection and Food Safety		Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.
Germany	D, 12 Environmental Monitoring Plan	Federal Office of Consumer Protection and Food Safety	1/ The monitoring plan is basically acceptable, but needs further elaboration for implementation. Therefore, the applicant is recommended to revise the monitoring plan during the initial implementation phase (after consent is given) and present this revised monitoring plan together with a first report one year after consent is given to be re-assessed. 2/ The risk assessment of MON 87460 can not be finalized because of deficiencies of the application listed above. Therefore the monitoring plan concerning the Case Specific Monitoring may need to be revised depending on the results of an updated risk assessment. 3/ The strategy of General Surveillance is mainly based on the involvement of importers, traders, silo operators and processors coordinated by EuropaBio. The applicant will inform the selected networks of operators about market release of GM plant products und will remind them to report on 'any unanticipated adverse effect'. It is stated that these third parties have to follow legal obligations of food and feed hygiene (HACCP). Nevertheless, the role and interplay of all actors on behalf of recording, analysis, and evaluation of monitoring data need more transparency. Additionally, other sources of information, e.g. peer-reviewed publications, should be taken into account. The monitoring plan does not relate the monitoring activities	1/ EFSA reiterates that monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gave its opinion on the scientific content of the monitoring plan provided by the applicant, and considered that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. 2/ As the scope of the application EFSA-GMO-NL-2009-70 does not include cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable grains of maize MON 87460 during transport and processing for food and feed uses, and with the exposure through manure and faeces from animals fed maize MON 87460 grains. The environmental risk assessment identified no potential adverse effects to the environment. Therefore, the EFSA GMO Panel concluded that no case-specific monitoring is necessary. 3/ The general surveillance plan proposed by the applicant includes: (1) the description of an approach involving operators (federations involved in maize import and

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
			to relevant protection goals. Even more it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter, because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered and how they will be evaluated. In addition, it might be useful to integrate food and feed surveillance in coordination with the competent authorities. Information about the use of the product in food and feed could deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Furthermore, the applicant should specify monitoring activities in the field of human and animal health. Therefore, it should be described in more detail how animal and human health surveillance is integrated in the monitoring plan. A report on GS activities on an annual basis is sufficient. Joint reports considering different approved GM plant products are acceptable, but it has to be guaranteed that each specific event is evaluated per se.	processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by EuropaBio for the collection of the information recorded by the various operators; and (3) the use of networks of existing surveillance systems (Lecoq et al., 2007; Windels et al., 2008). The applicant proposed to submit a general surveillance report on an annual basis and a final report at the end of the consent period. The EFSA GMO Panel considered that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460, as the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. Lecoq E, Holt K, Janssens J, Legris G, Pleysier A, Tinland B and Wandelt C, 2007. General surveillance: Roles and responsibilities the industry view. Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 2(S1), 25-28 Windels P, Alcalde E, Lecoq E, Legris G, Pleysier A, Tinland B and Wandelt C, 2008. General Surveillance for Import and Processing: the EuropaBio approach. Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety, 3(S2), 14-16.
Italia	General comments	Ministero dell'Ambiente e	Regarding the application for authorization in EU of MON 87460 maize, in the dossier there is an inadequacy in the information about molecular characterization, field experiments, allergy tests. Comments are detailed below in the appropriate fields.	A general comment is made by Italy; no specific question is posed to the EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		della Tutela del Territorio e del Mare		
Italia	D.Information relating to the GM plant	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	In our opinion the methodology used for the genetic modification is inadequate for two major reasons: (1). the choice to use a constitutive promoter to drive the cspB gene expression; and (2) the use of nptII cassette	The EFSA GMO Panel considered that the choice of this promoter does not raise a safety concern. With regard to the *npt*II gene, the EFSA GMO Panel requested further information concerning the presence of the *npt*II gene and the possibility for horizontal gene transfer. Please see the scientific opinion and additional information provided on 04/10/2010 and 30/04/2012 for further details.
Italia	D, 01 Description of the trait(s) and characteristics which have been introduced	Ministero dell'Ambiente e della Tutela del Territorio e del	As described in the dossier (page 4, Technical Dossier), proteins similar to cspB exist in plants, they bind RNA, unfold RNA secondary structures caused by environmental stress, and help to maintain cellular functions under stress conditions. The constitutive expression of the bacterial cspB gene should cause unpredictable effects on the cellular metabolism. The applicant provides no data nor makes any comment about the possibility that the presence of the protein CspB of bacterial origin could affect expression of this class of proteins. For example, csp 310 has been characterized in maize and other cereals. It is involved in plant protection under low temperature stress (1,2,3). It could be useful to analyse the behaviour of this protein in	The EFSA GMO Panel considered that the outcomes of the comparative assessment of agronomic, phenotypic, and compositional endpoints did not indicate the occurrence of unintended effects that would cause safety concerns.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Mare	the presence of cspB . 1. Non-phosphorylating bypass of the plant mitochondrial respiratory chain by stress protein CSP 310. Kolesnichenko AV, Grabelnych OI, Pobezhimova TP, Voinikov VK. Planta. 2005 Apr;221(1):113-22. Epub 2005 Jan 25. 2. Influence of CSP 310 and CSP 310-like proteins from cereals on mitochondrial energetic activity and lipid peroxidation in vitro and in vivo. Kolesnichenko AV, Zykova VV, Grabelnych OI, Koroleva NA, Pobezhimova TP, Konstantinov YM, Voinikov VK. BMC Plant Biol. 2001;1:1. Epub 2001 Sep 26. 3. Stress protein CSP 310 causes oxidation and phosphorylation uncoupling during low-temperature stress only in cereal but not in dycotyledon mitochondria. Grabelnych OI, Pobezhimova TP, Kolesnichenko AV, Voinikov VK. J Immunoassay Immunochem. 2001;22(3):275-87.	
Italia	D, 02 Information on the sequences actually inserted or deleted	Ministero dell'Ambiente e della Tutela del Territorio e del Mare	Technical Dossier, page 36: regarding the lox sites, is not clear if the applicant intend to remove the region between them, and if yes why has not done it in MON87460 yet.	The GM plant has been risk assessed by the EFSA GMO Panel as it was presented by the applicant (including presence of *npt*II gene and lox sites.
Italia	D, 07.02 Field trials	Ministero dell'A	- The notifier gives no information respect to the FAO class line of LH19 genetically modified with MON 87460, the isogenic lines used as control and the majority of reference varieties.	At the request of the EFSA GMO Panel, the applicant provided additional information (October 2010) on the breeding pedigree of maize MON 87460 and its comparators

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		mbiente della Tutela del Territorio e del Mare	- Moreover, as control are used two different lines, in different years and in different test sites - Most varieties of control is not listed in any catalog or list, while, among the few varieties present, some are expired and some others are rejected in Europe	used in the various comparative studies (for an overview, see Table 2), and confirmed that the comparators used had a comparable genetic background with maize MON 87460. Therefore, they can be regarded as suitable conventional counterparts.
Norway	General comments	The Norwegian Directorate for Nature Management	The Norwegian CA request the Notifier to list all third countries where applications MON 87460 have been, or is known to be, submitted. The list should include scopes of the applications and regulatory status in the individual third countries. The Norwegian CA sees this as important information in order to collect relevant information for the risk assessment of MON 87460.	Not in the remit of the EFSA GMO Panel.
Norway	General comments	The Norwegian Directorate for Nature Manageme	The Norwegian CA requests the Notifier to provide further information that will allow the Norwegian authorities to evaluate the possible contributions of maize MON 87460 to a sustainable development, benefit to the society and other ethical considerations regarding the use of the genetically modified crop. These aspects will be addressed in the evaluation of the notification in Norway under the Norwegian Gene Technology Act and in accordance with the Regulations relating to impact assessment pursuant to the Gene Technology Act (http://www.regjeringen.no/nb/dep/md/dok/lover_regler/forskrifter/2	Not in the remit of the EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		nt	005/regulations-relating-to-impact-assessmen.html?id=440455)	
Norway	D, 01 Description of the trait(s) and characteristics which have been introduced	The Norwegian Directorate for Nature Management	MON 87460 was developed to provide reduced yield loss under water-limited conditions compared to conventional maize. The Norwegian CA is in principle positive to the drought-resistance trait, if the trait proves to inter alia have beneficial impact on agronomical yield and on rural communities' economical income. The technical dossier contains some information on the agronomical yield, and the presented data suggests no significant yield advantage under well-watered conditions, but a certain yield advantage for MON 87460 under water limited conditions compared to a control. However, as the Notifier emphasizes, MON 87460 yields will be reduced to zero under severe water deficit. We request the applicant to 1) elaborate on under which drought stress levels MON 87460 is expected to outperform a control with similar genetic background, 2) discuss whether a 10-20 % agricultural yield gain is sufficient to balance the premium the farmers will have to pay for MON 87460 seeds, 3) discuss whether the yield gain could be achieved through conventional breeding with the control as breeding material, and 4) elaborate on the yield performance of MON 87460 under water limited conditions compared to conventional drought tolerant varieties.	Based on the dataset provided by the applicant, the EFSA GMO Panel concluded that under water-limited conditions, maize MON 87460 exhibited lower yields than in well-watered conditions but higher yields across locations compared with its conventional counterpart, though these differences were not consistently observed across studies and seasons. The remaining questions posed by Norway are not in the remit of the EFSA GMO Panel.
Norway	D, 05 Genetic stability of the insert and phenotypic stability of	The Norwegian Directorate for Natur	The Notifier has demonstrated stability of the inserted DNA sequence over generations by Southern blots. The stability of the insert is critical inter alia for traceability of the GMO. The Norwegian CA notes that publications describe single nucleotide polymorphisms (SNPs) in commercial GMOs as compared to the sequences described in applications. In Morisset et al. 2009 a commonly used screening method for P35s showed a 16-fold lower sensitivity than another	Not in the remit of EFSA GMO Panel.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
	the GM plant	e Management	screening method when analyzing TC1507 GM maize certified reference material (CRM). According to the authors this reduced sensitivity was due to a SNP in the target region of qPCR method. The SNP was not described in the dossier, patents or in other P35s sequences inserted into transgenic plants. We request the applicant to demonstrate the sensitivity of commonly applied PCR-based screening methods detecting genetic elements present in MON 87460 using MON 87460 CRM. Reference: Morisset,D., Demsar,T., Gruden,K., Vojvoda,J., Stebih,D., Zel,J. (2009). Detection of genetically modified organisms-closing the gaps. Nature Biotechnology 27, 700-701.	
Norway	C, 03 Size, source (name) of donor organism(s) and intended function of each	Norwegian Scientific Committee for Food Safety	The nptII expression cassette in maize line MON 87460 contains the coding sequence of the npt II gene from E. coli, which is flanked by two functional loxP sites. The loxP recombination site is recognized by the P1 bacteriophage Cre recombinase. The Cre/loxP site-specific recombination system has been applied in various plant species for marker gene removal, and according to the applicant the lox-P sites in MON 87460 were inserted to facilitate the potential excision of the nptII cassette, specifically using Cre recombinase. However, referring to the lack of safety concerns around the NPTII protein, the applicant concludes that it was unnecessary to excise the nptII gene using the loxP sites that flank this gene. The applicant is therefore asked to clarify why the loxP sites have been added to MON 87460.	The EFSA GMO Panel requested further information concerning the presence of the *npt*II gene and the possibility for horizontal gene transfer. Please see the scientific opinion and additional information provided on 04/10/2010 and 30/04/2012 for further details.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Norway	D, 01 Description of the trait(s) and characteristics which have been introduced	Norwegian Scientific Committee for Food Safety	The ability of MON87460 to tolerate drought stress is conferred by expression of the cspB gene from B. subtilis. CspB belongs to the bacterial cold shock protein (CSP) family, which is a group of small proteins characterised by the presence of a highly conserved RNA-binding sequence identified as cold shock domain (CSD). According to the applicant, the CspB protein is suggested to function as a "RNA-chaperone". CSPs recognize single stranded polynucleotides without apparent sequence specificity and facilitate the initiation of translation by destabilizing non-productive secondary structures in mRNA under environmental stress. Since the exact mechanism of drought tolerance in MON87460 maize remains to be defined, and due to the apparent absence of binding sequence specificity indicating that plant CSD-containing proteins could be involved in a more general response to stress by binding RNAs, we would like the applicant to discuss the possibility that other physiological processes in the plant may be affected by the expression of the cspB gene.	Extensive description of the underlying mechanisms was not considered needed by the EFSA GMO Panel given that phenotypic, agronomic and compositional analyses have not identified unintended effects.
Norway	D, 06 Any change to the ability of the GM plant to transfer genetic material to	Norwegian Scientific Committee for Food Safety	The microbial flora in the gastrointestinal tract of cattle may contain bacteria harbouring the bacteriphage P1 and the Cre-recombinase. Aminoglycoside antibiotics are to some extent used to treat gastrointestinal infection in cattle, and therefore the presence of these antibiotics will make a selective pressure which will facilitate a transfer of the nptII cassette to gastrointestinal bacteria.Since the lox sites and intervening sequences are present in the GM variety, the putative horizontal spread of the transgenes in the intervening sequence has to be evaluated.	The EFSA GMO Panel considered that the stabilisation of the *loxP-npt*II-*loxP* fragment due to the Cre recombination system present in bacteria containing a P1 or P1-like bacteriophage is unlikely.
Norway	D, 07.08 Toxicology	Norwegian Scientific	In reference to 7.8.4, 7.10.2, MSL0021408-2008 and WI-2007-064 the objective of the 42-days study and the 90 days study was to evaluate the nutritional value and potential health effects of broiler and rats fed diets containing processed corn grain from MON 87460	According to EFSA GMO Panel guidelines, the testing with whole foods is not a mandatory requirement. The outcomes of the comparative assessment would not have triggered a request for such animal studies.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Committee for Food Safety	compared to grain from a conventional control variety. However, it is not evident from the dossier whether corn from MON 87460 plants exposed to stress conditions has been used in these studies. The Norwegian GMO Panel is of the opinion that the 42 days broiler study and the 90 days rat study also should include feed with grain from MON 87460 plants produced under water-limited conditions. The applicant is also asked to clarify why the acute toxicity test in mice (with pure protein) has been carried out with a low protein dose (4.7 mg/kg bodyweight). According to the OECD guideline 401 "Acute oral toxicity", a limit test at one dose level of at least 2000 mg/kg bodyweight may be carried out. The Norwegian GMO Panel is of the opinion that the applicant, in order to exclude any acute health effects of CspB protein, should have performed an acute toxicity test on mice with at least 2000 mg/kg bodyweight with purified CspB protein.	The characteristics of the maize grains of test and control maize used for dietary preparation and the resulting diets are provided in the appendices B-E on pages 451-483 of the report on the 90-day rat feeding study (WI-2007-064 2008). WI-2007-064 (2008) A 90-day feeding study in rats with drought tolerant corn: MON 87460. Study Number WIL-50342. Sponsor Study Number WI-2007-064. Wil Research Laboratories for Monsanto, St. Louis, USA [part of confidential dossier information]
Spain	D.Information relating to the GM plant	Ministry of the Environment, and Rural and Marine Affairs	The molecular characterisation is complete (insert and associated flanking sequences) and the results can be considered satisfactory, although the CNB noted that the Southern analyses carried out with three overlapping probes it is not considered as an appropriate scientific methodology.	In this case, the EFSA GMO Panel requested the applicant to repeat the Southern analyses using separate probes to cover the vector backbone sequence. The applicant provided new Southern blot analyses in Song (2010). Song, Z., et al. (2010) Confirmation of the absence of plasmid vector PV-ZMAP595 backbone sequence in the genome of MON 87460 by Southern Blot analysis. Report RAR-10-282. Monsanto Co., St. Louis, USA [as part of confidential dossier information, additional information received on 4 October 2010]

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Spain	D, 07.08 Toxicology	Ministry of the Environment, and Rural and Marine Affairs	In this case, the notifier presents a 90-day sub-chronic toxicity study with the MON 87460 maize grains in rats, and the results indicate that no significant toxic effects are observed. Therefore, in our opinion no further studies are necessary.	The EFSA GMO Panel appreciated this conclusion of Spain.

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
Spain	D, 12 Environmental Monitoring Plan	Ministry of the Environment, and Rural and Marine Affairs	1/ This application is only for authorisation for import, processing, and food and feed uses, but not for cultivation. However, the measures to avoid accidental spillage should be strengthened. 2/ The consent holder provide details of the arrangements of the monitoring plan, in particular for general surveillance, indicating which existing network programs could be used, the type of information that should be collected and a more detailed monitoring methodology in order to have a monitoring plan which could be implemented in a harmonised manner among the importer Member States. 3/ The important thing at a later stage will be to evaluate how the general surveillance it is implemented and controlled at national and European level through the nets of operators proposed by the notifier to detect unforeseen potential adverse effects and the accidental spillage into the environment and their consequences, as well as to tackle issues as the information transmission among the operators with the Competent Authorities, and the assessment of the annual reports given by the holder of the product and foreseen in the own monitoring plan.	1/ The EFSA GMO Panel acknowledged the approach proposed by the applicant to put in place appropriate management systems to restrict environmental exposure in the case of accidental release of viable grains of maize MON 87460. Considering the intended uses of maize MON 87460 and the physical characteristics of maize seeds, possible pathways of gene dispersal are (accidental) grain spillage during transport and processing and the dispersal of pollen from occasional feral GM maize plants originating from grain spillage. Overall, the EFSA GMO Panel considered that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 87460 will not differ from that of conventional maize varieties. 2/ EFSA reiterates that monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gave its opinion on the scientific content of the monitoring plan provided by the applicant, and considered that the scope of the monitoring plan provided by the applicant is in line with the intended uses of maize MON 87460. 3/ Not in the remit of the EFSA GMO Panel.
The Netherlands	D, 07.01 Comparative assessment	Ministry of Agriculture,	The raised levels abscisic acid in forage observed in the study of Alba et al 2008 in two samples well watered MON87460 forage from one Chilean site and one (outlier) level at another site are not well explained. The applicant should provide further data on variability of abscisic level in forage from maize grown under well watered and	The applicant identified that "*Only the abscisic acid value in MON 87460 forage obtained from site CT was considered a true outlier (PRESS Std Residual > 7) and removed from further analysis*." and concluded that "*The other two mean component values observed to be significantly different*

Application EFSA-GMO-NL-2009-70 (MON 87460 maize Monsanto) Comments and opinions submitted by Member States during the three-month consultation period				
Comments from National Competent Authorities under Directive 2001/18/EC				
Country	**Reference**	**Organization**	**Comment**	**ANNEX G**
		Nature and Food Quality and Ministry of Health	water limited conditions, to exclude the possibility that these abscisic acid levels are an indication for (other) unintended effects in the physiology of the MON87460 maize plants.	*between test and control fell outside the 99% tolerance interval and both were for abscisic acid (one from the combined-site analysis and another from the CL site). The raw data shows that these statistically significant differences originate from two replicates of MON 87460 at the CL site. The forage tissue from these two replicates had levels of abscisic acid (85.2 ppb, 122 ppb) that were 4.5 to 7.5 times greater than the overall mean of this metabolite in forage. Forage from the third replicate from the CL site contained 18.5 ppb abscisic acid, which is nearly identical to the overall mean of this metabolite in forage from the CL, CT, and LUM sites. Thus, the levels of abscisic acid in forage at the CL site were extremely variable*." (Alba et al 2008).