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Austria	Federal Ministry of Health	General comments	The present notification repeatedly makes reference to preceding applications for the parental single events - GM maize MON88017 (EFSA/GMO/CZ/2005/27), MON89034 (EFSA/GMO/NL/2007/37), 1507 (EFSA/GMO/NL/2004/02) and 59122 (EFSA/GMO/NL/2005/12). A number of concerns have been raised towards the conclusiveness of data submitted in the context of these notifications, some of which have not been addressed by additional information submitted by the notifiers. Therefore, it is requested that these concerns are addressed by the notifier in the course of evaluation of the present notification by submission of specific information on GM maize MON88017xMON89034x1507x59122. A stacked organism has to be regarded as a new event, even if no new modifications have been introduced. The gene-cassette combination is new and only minor conclusions could be drawn from the assessment of the parental lines, since unexpected effects (e.g. synergistic effects of the newly introduced proteins) cannot automatically be excluded. Furthermore, it should not be neglected that two of the parental lines, GM maize MON89034 and GM maize MON88017, have not yet gained authorisation within the European Union.	The single events 1507, 59122, MON 88017 and MON 89034 have been the subjects of previous assessments and have received an EFSA opinion in favour of their authorisation (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b). All single events have been authorised for food and feed, import and processing in the EU (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm) In case of this stack the applicant is requested to follow the Guidance Document of the EFSA Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events (2007)

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Austria	Federal Ministry of Health	General comments	Detection Method: Providing of four event specific detection methods for each parental line and an maize-specific reference PCR system is not satisfactory in this respect. Generally, a validated event specific detection method for the stacked event should be presented before deciding about the placing on the market of this product. Such an event specific detection method would be, for instance, a validated multiplex PCR where in a single assay all four targets are detected simultaneously. Furthermore, as long as no official (guidance) document on the interpretation of detection results, i.e. how to distinguish between a stacked event and its respective single events, of the described method for stacked events is available, no approval for placing on the market of this product should be given. Even the notifier gives clear indication of this problem, "Given that MON89034×1507×MON88017×59122 would be indistinguishable from a combination of MON89034, 1507, MON88017 and 59122 in mixed consignments of maize products, certified reference materials of the parental products are considered appropriate for MON89034x1507×MON88017×59122" (see Part V).	Outside the remit of the EFSA GMO Panel.
Austria	Federal Ministry of Health	D, 02 Information on the sequences actually inserted or deleted	The data submitted for molecular characterisation of GM maize MON88017xMON89034x1507x59122 consist of southern blots to demonstrate the presence of the introduced traits (Cry1A.105, Cry2Ab2, Cry3Bb1, Cry1F, Cry34Ab1, Cry35Ab1, pat and epsps) by comparative analysis with the parental single events. These data however are not entirely sufficient to demonstrate that the structure of the inserts is conserved, and that the likelihood for changes due to interaction of transgenic elements by recombination is low. No information is submitted by the notifier with regard to the fact how many individual plants were screened to determine the degree of stability in GM maize MON88017xMON89034x1507x59122. Since the inserts introduced into GM maize MON88017xMON89034x1507x59122 from different parental events contain a number of similar genetic elements (among others sequences from the 35S-promoter in all inserts, and sequences from the ubiquitin promoter, the rac intron, the nos-terminator, the 35S-terminator and the pat gene in two inserts	Southern analyses demonstrated that the structure of all inserts was retained in the stack. The molecular data supplied by the applicants do not suggest a structural modification due to the traditional breeding methods used to obtain the stack. The stability of the single events was determined over several generations, stability of the stack over one generation. This is in line with the EFSA guidance document (2007) and is considered to be sufficient from a safety point of view. In the view of the EFSA GMO Panel, additional information concerning recombination is not routinely needed to carry out the risk assessment. Unless inserts are located extremely close on the same chromosome (within kilo base range), no homologous recombination is expected to occur between them. Furthermore, this maize stacked line

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			each) homologous recombination events between the inserts cannot be ruled out. The notifier, thus, is requested to assess the stability of the inserts and the probability that the structure of the inserts is retained. Furthermore, only one of the studies submitted by the notifier (Schafer et al. 2008) identifies the maize line which was used as non-transgenic control (XE6001 which according to the pedigree information (technical dossier, p. 51) is a maize line with a similar genetic background to GM maize MON88017xMON89034x1507x59122. The other study submitted on the comparison of GM maize MON88017xMON89034x1507x59122 with MON88017 and MON89034 (Taylor et al. 2007) does not identify the used control material beyond that it was regarded as "conventional" maize. Thus, the notifier is requested to identify all used control lines and justify their appropriateness. Furthermore, all concerns raised by Austria towards the molecular characterisation of the individual parental events - GM maize MON88017 (EFSA/GMO/CZ/2005/27), MON89034 (EFSA/GMO/NL/2007/37), 1507 (EFSA/GMO/NL/2004/02) and 59122 (EFSA/GMO/NL/2005/12) should be addressed by the notifier in the context of the present notification. [Schafer, B. W., Cai, C. Q. and Embrey, S. K. (2008). Southern Blot Analysis to Confirm the Presence of TC1507 and DAS-59122-7 in the Combined Trait Corn Product MON 89034 x TC1507 x MON 88017 x DAS-59122-7. Dossier EFSA/GMO/CZ/2008/62, Regulatory Sciences and Government Affairs - Indianapolis Lab / Dow AgroSciences LLC.] [Taylor, J. P., Groat, J. R. and Masucci, J. D. (2007). Southern Blot Analyses to Confirm the Presence of MON 89034 and MON 88017 in the Combined Corn Trait Product MON 89034 × TC1507 × MON 88017 × DAS-59122-7. Dossier EFSA/GMO/CZ/2008/62, Monsanto Company.]	is only grown for one generation therefore the analysis should be carried out on this generation. The agronomic characteristics of MON 89034 x 1507 x MON 88017 x 59122 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns. The study of Taylor *et al* (2007) gives a direct comparison of GM maize MON 89034 x 1507 x MON 88017 x 59122 with the single events MON 88017 and MON 89034 to confirm the presence of both events in the stacked line. These blots include also a conventional maize control spiked with one copy of plasmids PV-ZMIR245 and ZMIR39 (positive control). Data confirm the presence of both events in the stacked line. No unexpected hybridisation signals were observed. The identity of the conventional control is therefore not relevant for the conclusion. Concerning the issues raised on the single events we refer to the respective Annexes G from the opinions (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b)
Austria	Federal Ministry of Health	D, 03 Information on the expression	The notifier presents expression data for the transgenic proteins contained in GM maize MON88017xMON89034x1507x59122 (Stillwell and Silvanovich 2007; Phillips 2008) in materials from field tests conducted 2006 in the USA (Monsanto Company and Dow	Expression levels of recombinant proteins in maize plants with or without treatment of herbicides were previously assessed for the single events and do not have to be repeated for the stacked lines. Moreover,

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		of the insert	AgroSciences LLC 2007). The information submitted by the notifier does not assess the expression of the transgenic proteins in plants not treated with herbicides containing glyphosate and glufosinate-ammonium. According to the EFSA guidance how to conduct field trials for comparative assessment, "in case of herbicide-tolerant GM-plants it is advisable to include both blocks of genetically modified plants exposed to the intended herbicide and blocks not exposed to the herbicide. This design would allow assessment of whether the expected agricultural conditionmight influence the expression of the studied parameters" (EFSA 2006). Since such a comparison between expression of transgenic components in treated vs. untreated plants was not conducted, the submitted information is considered insufficient. Additional information addressing this issue is requested from the notifier in line with EFSA guidance. Furthermore, expression is only assessed for a single growing season. An assessment of expression over several growing seasons would be more adequate to establish baseline exposure data. The notifier, thus, is requested to present data from at least 2 consecutive growing seasons. [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.] [Monsanto Company and Dow AgroSciences LLC (2007). A U.S. Field Production of Corn Grain and Tissues from NK603, MON 89034, TC1507, MON 88017, DAS-59122-7, Conventional Crosses MON 88017 × DAS-59122-7, MON 89034 × MON 88017 × DAS-59122-7, MON 89034 × TC1507 × NK603, MON 89034 × TC1507 × MON 88017 × DAS-59122-7, and a Conventional Control During 2006. Dossier EFSA/GMO/CZ/2008/62.] [Phillips, A. M. (2008). Cry34Ab1, Cry35Ab1, Cry1F and PAT Protein Levels in Hybrid Maize TC1507, DAS-59122-7, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a Conventional Control from the	only grains from treated plants will be imported. In addition, none of the newly expressed proteins is considered to be toxic to the consumers. Expression levels of the single events were already assessed. For stacked lines expression data for one season are considered to be sufficient.

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			Monsanto 2006 Production Plan 06-01-52-04. Dossier EFSA/GMO/CZ/2008/62, Regulatory Laboratories - Indianapolis Lab / Dow AgroSciences LLC.] [Stillwell, L. and Silvanovich, A. (2007). Assessment of Cry1A.105, Cry2Ab2, Cry3Bb1, and CP4 EPSPS Protein Levels in the Combined Trait Corn Product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in U.S. Field Trials During 2006. Dossier EFSA/GMO/CZ/2008/62, Monsanto Company.]	
Austria	Federal Ministry of Health	D, 03 Information on the expression of the insert	D.3. (c) Expression of potential fusion proteins: The notification does not contain a specific discussion of the expression of potential fusion proteins in GM maize MON88017xMON89034x1507x59122, but makes reference to the information submitted in the context of the notifications on the individual parental events. Therefore, any concerns raised towards the assessment of the individual parental events - GM maize MON88017 (EFSA/GMO/CZ/2005/27), MON89034 (EFSA/GMO/NL/2007/37), 1507 (EFSA/GMO/NL/2004/02) and 59122 (EFSA/GMO/NL/2005/12) should be addressed by the notifier in the context of the present notification. Specifically, the assessment should be based on recommendations for the assessment of potential homologies to allergens (FAO/WHO 2001). In line with these recommendations a 6-mer search should be conducted for the assessment of the allergenic potential of any identified potential fusion proteins. Additionally, the theoretical analysis by homology comparisons should be supported by experimental data for relevant ORFs, which might be expressed as fusion proteins. The notifier is requested to submit additional evidence and information addressing the mentioned concerns. [FAO/WHO (2001). Evaluation of Allergenicity of Genetically Modified Foods - Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology.]	The presence and integrity of each of the inserts of the single events (including the flanking regions) was demonstrated in GM maize MON 89034 x 1507 x MON 88017 x 59122. There is no reason to assume that fusion proteins in GM maize MON 89034 x 1507 x MON 88017 x 59122 will differ from those in the single events. A reference to data of the single events is therefore sufficient. Updated bioinformatic analyses were provided for all novel open reading frames (ORFs) in the insert/plant junction regions for all events. Similarity of all these ORFs with known allergens was determined. According to the guidelines (*Codex alimentarius*, 2003) the novel ORF sequences were screened for any 8 identical amino acid matches with known allergens. Also a 35% or greater identity threshold over any 80 or greater amino acid sequences between the query protein and an allergen was used to indicate the allergenicity potential. Using this approach, none of the novel ORFs showed significant similarity with known allergens. Therefore, the EFSA GMO Panel considers this sufficient from a safety point of view.

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Austria	Federal Ministry of Health	D, 04 Information on how the GM plant differs from the recipient plant in:	The conclusions of the notifier with respect to the phenotypic and ecological characteristics of GM maize MON88017xMON89034x1507x59122 are based on the data established for the parental single events and data from field trialling in the USA (Rosenbaum 2008). The phenotypic and ecological observations made in the field trials do not indicate differences between GM maize MON88017xMON89034x1507x59122 and the control. However, the phenotypic characteristics evaluated in the field trial merely comprise standard agronomic parameters as used by plant breeders and agronomists. Ecologically important characteristics as for instance the flowering time, pollen size and production or the duration of pollen viability have not been assessed. For the ecological evaluation the small size of the field trial plots may limit the significance of the results. The assessment of ecological behavior should furthermore be addressed with a specific design of the trial to establish the occurrence of certain environmental conditions during the field trial, e.g. the demonstration of the occurrence of the respective pest or disease in the growing area (testing under pest/disease pressure) in order to assess the susceptibility to pests and diseases. The experimental design did not include plots which were not treated with the non-selective herbicides (glyphosate and glufosinate-ammonium), and thus, did not allow for a comparison between treated and untreated plots. To address the abovementioned concerns the notifier should submit further data on ecologically relevant parameters such as duration of pollen viability, flowering time or susceptibility towards pest and disease (investigated under pest or disease pressure). [Rosenbaum, E. W. (2008). Phenotypic Evaluation and Ecological Interactions of the Combined Trait Corn Product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Grown During 2006. Dossier EFSA/GMO/CZ/2008/62, Monsanto Company.]	The EFSA GMO Panel thanks Austria for drawing its attention to this detail in the comparative analysis. In section 4.1.2 describing the field trial design for the comparative analysis, the EFSA GMO Panel's opinion addresses the issue of herbicide treatment with the target herbicides as follows: "*Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides).*" The herbicide treatment is described in more detail in the appendix production plan 06-01-52-04. In addition, in response to a query by the EFSA GMO Panel, the applicants have explained that the doses of glufosinate-ammonium and glyphosate-based herbicides are representative of those used in commercial practice. The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from

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				agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity. Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON 89034 x 1507 x MON 88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events

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				and maize stacks (1507 x 59122, MON 89034 x MON 88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 x 1507 x MON 88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from that of conventional maize varieties.
Austria	Federal Ministry of Health	D, 04 Information on how the GM plant differs from	Phenotypic Evaluation and Ecological Interactions (Rosenbaum 2008): a) General comments: Because of the lack of raw (individual) data on all phenotypic parameters it cannot be concluded that there are no relevant	It is noted by the EFSA GMO Panel that the field trials performed for the comparative analysis need not necessarily comply with the requirements for variety testing given that the purpose of the latter (e.g. demonstration of a certain difference with the

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		the recipient plant in:	differences between test substance (MON88017x1507xMON88017x59122) and control substance (XE6001). Providing of the raw data in electronic form would be appreciated in order to reproduce the reasoning of the study. Due to little disease pressure at the sites no real evaluation of the different substances (control, test, reference) is possible, especially with regard to the efficacy of the insect resistance traits. Although the authors of the study noted once, "The purpose of this study was to assess the phenotypic characteristics and ecological interactions of the combined trait corn products MON89034x1507xNK603 (triple stack) and MON89034x1507xMON 88017x59122 compared to a conventional corn control", this respective triple stack is not again mentioned in the whole paper. although it would be interesting to know how the assessment of the triple stack was implemented in the randomised complete block design in order to allow better judging of the validity of the whole study. b) Study design: Use of randomised complete block (RCB) design with three replications and the use of SAS® analysis software, as well as the length and width of the plots, are in accordance with modern standards as used by plant breeders and agronomists. However, with respect to the ecological evaluation, the small size of the field trial plots may limit the significance of the results. Comparing the study design with legal standards for authorisation of new plant varieties in Austria (Republik Österreich 1997; AGES 2002), it should be mentioned that at least two years of testing at eight different sites is considered necessary to enable sound ecological assessment. From this point of view, a number of four sites seems to be rather low. Additionally, ecologically important characteristics as for instance the flowering time, pollen size and production or the duration of pollen viability have not been assessed.	comparators) is different from that of the comparative safety assessment. The EFSA GMO Panel's guidance on the assessment of stacked events thus mentions in section 3.2.2: "Possible differences in phenotypic characteristics and agronomic properties of stacks must be assessed in field trials over at least one season" In section 4.1.4 of its opinion, the EFSA GMO Panel comments on the outcomes of the study on agronomic/phenotypic characteristics as follows: "*In the present application, the analyses of agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and twelve commercial maize varieties included a range of parameters related to plant morphology, physiology, appearance and performance, including stressors and plant health. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize containing stack MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart but this was not consistently observed in each location."* In section 4.1.2 describing the field trial design for the comparative analysis, the EFSA GMO Panel's opinion addresses the issue of herbicide treatment with the target herbicides as follows: "*Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA*

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			The assessment of ecological behavior should, furthermore, be addressed with a specific design of the trial to establish the occurrence of certain environmental conditions during the field trial, e.g. the demonstration of the occurrence of the respective pest or disease in the growing area (testing under pest/disease pressure) in order to assess the susceptibility to pests and diseases. We are, therefore, of the opinion that the notifier should submit further data on ecologically relevant parameters (e.g. duration of pollen viability, flowering time or susceptibility towards pest and disease (investigated under pest or disease pressure). Moreover, the experimental design did not include plots which were not treated with the non-selective herbicides (glyphosate and glufosinate-ammonium), and thus, did not allow for a comparison between treated and untreated plots. [AGES (2002). Sorten- und Saatgutblatt, 10. Jahrgang. Richtlinien für die Sortenprüfung - Methoden für Saatgut und Sorten.] [Republik Österreich (1997). Saatgutgesetz, BGBl. I Nr. 72/1997 in der Fassung BGBl. I Nr. 109/2001.] [Rosenbaum, E. W. (2008). Phenotypic Evaluation and Ecological Interactions of the Combined Trait Corn Product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Grown During 2006. Dossier EFSA/GMO/CZ/2008/62, Monsanto Company.]	*GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides).*" The herbicide treatment is described in more detail in the appendix production plan 06-01-52-04. In addition, in response to a query by the EFSA GMO Panel, the applicants have explained that the doses of glufosinate-ammonium and glyphosate-based herbicides are representative of those used in commercial practice. The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity.

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				Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON 89034 x 1507 x MON 88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON 89034 x MON 88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 x 1507 x MON 88017 x 59122, or maize with comparable properties such as single events

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				and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from that of conventional maize varieties.
Austria	Federal Ministry of Health	D, 04 Information on how the GM plant differs from the recipient plant in:	Phenotypic Evaluation and Ecological Interactions (Rosenbaum 2008) [cont.]: c) Definitions of characteristics: Definitions of some plant characteristics seem to be incomprehensible and misleading: - "Grain moisture: Percent moisture of shelled grain at harvest or after drying." In fact, provided data can only represent percent moisture at harvest. It would really make no sense to collect data on moisture contents after drying procedures. - "Test weight: Weight of shelled grain per unit volume at harvest or after drying." Again this is not understandable. - "Yield: Calculated from grain weight, moisture, and test weight". It is not clear how the yield was calculated, and it is also not clear how much	Table 6 of the appendix “production plan 06-01-52-04” mentions that grains harvested at the R6 stage were dried down to 12-15% moisture. With regard to drying practices in more general terms, it is noted by the EFSA GMO Panel that drying grains after harvest down to a lower moisture level (*i.e.* not completely dry) appears to be common practice in commercial agriculture, particularly if the harvest takes place during a wet/moist period leading to a high moisture content of the grain, in order to prolong the storability of the harvested grain (*e.g.,* grain with a high moisture content can only be stored during a very short period). Data on herbicide applications are provided in Table 5 of the appendix production plan 06-01-52-04, which also contains data on environmental factors

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			percent moisture was used as standard for this calculation. And, why and in what manner was the test weight included in the calculation, as the yield is totally independent of the test weight? d) Conducting the study: Obviously, singling and gapping after germination and emerging of the plants were not performed. This is a standard procedure in Austria field testing in order to guarantee homogeneity of the population. However, due to lack of raw data no conclusions can be drawn on the homogeneity of the populations (control, test, reference). Additionally, data on weed infestation and herbicide application are missing. e) Results: Few differences regarding the phenotypic characteristics were found between test and control substances for mean values across all sites. However, those statistically significant differences found for mean values at the different sites could have been caused by minimal environmental effects or minimal measuring errors. For instance, at site IL-1 the average plant height of the test substance was 11 centimetres higher than the control (105.5 / 101.2 inches). Pest pressure and infestation in general seemed to be negligible, as no or only little stressor symptoms were found only. Similarly, no abiotic stress through compaction, drought or frost was observed. As a consequence, statistical evaluation did not provide any differences between the test, the control and the reference substances. However, the reverse argumentation that actually there are no differences is not allowed. Furthermore, it is conspicuous that grain moisture is significantly higher (on average 1.2%) in the test substance MON88017x1507xMON88017x59122. In Austria the test substance would thus be rated as dent maize, i.e. 30 FAO units higher, which means later ripening. At one single site the difference for grain moisture was 3.1% (25.8 vs. 22.7), which would mean ca. 80 FAO units! In addition, higher values of staygreen (6.4), days to pollen shed (56.6)	and agronomic treatments. Table 6 of this appendix mentions that grains harvested at the R6 stage were dried down to 12-15% moisture. The report by Rosenbaum indicates which differences are statistically significant. In section 4.1.4 of its opinion, the EFSA GMO Panel comments on the outcomes of the study on agronomic/phenotypic characteristics, including the statistically significant differences observed in the across-location (no differences) and per-location statistical analyses, as follows: "*In the present application, the analyses of agronomic and phenotypic characteristics of maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and twelve commercial maize varieties included a range of parameters related to plant morphology, physiology, appearance and performance, including stressors and plant health. A number of parameters showed statistically significant differences in the per-location statistical analysis of the comparison between maize containing stack MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart but this was not consistently observed in each location.*" Concerning the apparent decrease in some leaf diseases at one location at a later stage that Austria points to, the EFSA GMO Panel notes that certain diseases proceed through various stages of plant infection (e.g. primary infection followed by secondary infection), which may also account for certain temporal variations in observed disease severity and progression, depending on environmental conditions (e.g. humid *versus* dry conditions).

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			and days to silking (55.7) were found. The yields though were considerably lower in the test substance (152.9 vs. 161.0)! According to the current standards for authorisation of new plant varieties in Austria (Republik Österreich 1997; AGES 2002), both a difference of 30 FAO units and lower yields of about 5% would be enough to refuse authorisation. However, this report does not give indication whether differences for grain moisture and yields are statistically significant, and due to missing raw data this cannot be verified. f) Other comments: Symptoms of the plant disease 'grey leaf spot' assumedly diminished between the 3rd and the 4th observation for all plant populations at the York site (NE). This is the same for the disease 'stressor rust'. The notifier is therefore requested to explain this behaviour. [AGES (2002). Sorten- und Saatgutblatt, 10. Jahrgang. Richtlinien für die Sortenprüfung - Methoden für Saatgut und Sorten.] [Republik Österreich (1997). Saatgutgesetz, BGBl. I Nr. 72/1997 in der Fassung BGBl. I Nr. 109/2001.]	The EFSA GMO Panel considers that the information provided in relation to the agronomic performance assessment is sufficient considering the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122, which excludes cultivation. In an environmental risk assessment, agronomic/phenotypic data give an indication on whether the fitness, persistence and invasiveness of the GM plant differ from that of its non-GM counterpart. Information on phenotypic and agronomic characteristics usually is obtained from agronomic field trials conducted at a range of locations representative of different environments where the GM crop may be grown. To assess the agronomic performance of the GM plant, different plant characteristics are recorded to establish differences between the GM plant and its non-GM counterpart. Fitness differences between the GM plant and its non-GM counterpart are usually inferred from a composite measure of relative plant germination, emergence, growth, survivorship, biomass and fecundity. Previous field trials have shown that there are no indications of altered fitness of the single maize events MON 89034, 1507, MON 88017 and 59122 and the two double stacks 1507 x 59122 and MON 89034 x MON 88017, as compared to their conventional counterparts. In addition to the field trials carried out with the single events and maize stacks (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b, c, 2010), a series of field trials with maize MON 89034 x 1507 x MON 88017 x 59122 was conducted at four locations within major maize-growing regions of the USA in 2006. Information on

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				phenotypic and agronomic characteristics was provided to assess the agronomic performance of maize MON 89034 x 1507 x MON 88017 x 59122 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of maize MON 89034 x 1507 x MON 88017 x 59122 plants, though there is a potential for enhanced biomass production when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests. On the basis of the available data on the single events and maize stacks (1507 x 59122, MON 89034 x MON 88017 and MON 89034 x 1507 x MON 88017 x 59122), the EFSA GMO Panel considers it very unlikely that the segregating progeny of MON 89034 x 1507 x MON 88017 x 59122 would have any increased persistence and invasiveness in EU receiving environments. In addition, the EFSA GMO Panel is not aware of any scientific report of increased establishment, spread or any change in survival capacity including overwintering of maize MON 89034 x 1507 x MON 88017 x 59122, or maize with comparable properties such as single events and sub-combinations of maize MON 89034 x 1507 x MON 88017 x 59122. Since maize MON 89034 x 1507 x MON 88017 x 59122 has no altered survival, multiplication or dissemination characteristics, except when glufosinate-ammonium- and/or glyphosate-based herbicides are applied and/or under infestation by target pests, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects due to the accidental release into the environment of viable grains from maize

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				MON 89034 x 1507 x MON 88017 x 59122 (which include all sub-combinations of the individual events) will not differ from that of the single maize events (MON 89034, 1507, MON 88017 and 59122), the two double stacks (1507 x 59122 and MON 89034 x MON 88017), or from that of conventional maize varieties.
Austria	Federal Ministry of Health	D, 05 Genetic stability of the insert and phenotypic stability of the GM plant	The genetic stability of GM maize MON88017xMON89034x1507x59122 was not assessed specifically by the notifier. Instead, the stability is inferred from respective information submitted for the individual parental events in preceding notifications - GM maize MON88017 (EFSA/GMO/CZ/2005/27), MON89034 (EFSA/GMO/NL/2007/37), 1507 (EFSA/GMO/NL/2004/02) and 59122 (EFSA/GMO/NL/2005/12). Furthermore, the notifier concludes that stability is not a concern because GM maize MON88017xMON89034x1507x59122 is produced by crossing of two transgenic parental lines and thus only exists for a single generation, with "no opportunity for its stability to be compromised" (technical dossier p. 62). However, the breeding pedigree shows that the two lines used for the production of GM maize MON88017xMON89034x1507x59122 are harboring pairs of transgenic inserts (MON88017xMON89034, and 1507x59122) derived from individual events and are propagated by selfing. The respective paired events do contain inserts harboring a number of similar genetic elements; thus, homologous recombination events between these inserts cannot be ruled out. Respective sequence elements are: sequences from the 35S-promoter, the rac intron and the nos-terminator in MON88017xMON89034; sequences from the 35S-promoter, the ubiquitin promoter, the 35S-terminator and the pat gene in 1507x59122. Therefore, the notifier is requested to submit additional information to address this issue. Specifically, an adequate number of individual plants should be analysed with methods which allow the assessment of the integrity of the transgenic insertions and the flanking sequences. Furthermore, the level of stability which can be detected by the experiments should be indicated.	The molecular data supplied by the applicants do not suggest a structural modification due to the traditional breeding of the single events of the stacked lines. The stability of the single events was determined over several generations, stability of the stacked event over one generation. This is considered to be sufficient from a safety point of view. The agronomic characteristics of MON 89034 x 1507 x MON 88017 x 59122 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns. Furthermore, the EFSA GMO Panel has assessed the parental stacks 1507 x 59122 (EFSA-GMO-UK-2005-15, EFSA 2009c) and MON 89034 x MON 88017 (EFSA-GMO-NL-2007-39, EFSA 2010) previously.

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Austria	Federal Ministry of Health	D, 07.01 Comparative assessment	Analysis of composition is based on data from trials conducted 2006 in the USA (Lundry et al. 2007). In the technical dossier, these data are represented in a very clear way, with an overview table and a table specifically presenting results of the compositional comparison, which indicate statistically significant differences between test and control line. However, the submitted data were determined in just one single growing season. A robust analysis should be conducted for more than one growing season at similar or comparable locations. Additionally, the test plots were treated with glyhosate- and glufosinate-containing herbicides during the trials. Apparently, no samples from GM maize MON88017xMON89034x1507x59122 not treated with these herbicides were analysed. No information is submitted whether an assessment of herbicide metabolites in treated plants was conducted. The notifier is, therefore, requested to submit additional data addressing the mentioned issues. Moreover, the trial results show significant differences for a number of analytes within locations and, for two analytes, even across locations. The notifier concludes that these differences are not biologically relevant, after comparison of the data with reference ranges obtained from the assessment of commercial maize lines grown at the test locations and with literature data. However, in a number of cases (e.g. vitamin B1 across locations, stearic acid at NE-site, folic acid at IL1-site) some of the data points were not within the indicated reference range (see technical dossier, tab. 21, p. 72-73). The notifier is requested to address these differences to clarify the underlying cause, as the assessment of compositional equivalence between the GM and the non-GM plant by itself is not considered to be a risk assessment in itself but rather the starting point for further assessments of a GM plant (Codex Alimentarius Commission 2003). [Codex Alimentarius Commission (2003). Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. Codex Alimentarius Commission, Joint FAO/WHO Food Standards	The EFSA GMO Panel's guidance on the assessment of stacked events, section 3.2.2, notes that "For the stacked events at least one year of field trial data is required, with trials performed together with appropriate controls in geographical localities representative of the climatic conditions under which such crops will be cultivated." It is noted that issues related to herbicide active ingredient kinetics and metabolism in the crop is a common feature of pesticide registration dossiers, which fall under the scope of parallel legislation, *i.e.* treated separately under the scope of Directive 91/414/EC and Regulation (EC) No 396/2005. With regard to the statistically significant differences observed in the comparison between maize MON 89034 x 1507 x MON 88017 x 59122, the EFSA GMO Panel's opinion notes the following: "*The outcomes of the comparison between maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart across locations showed that six parameters showed statistically significant differences in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122. Vitamin B1, oleic acid, and eicosenoic acid were slightly lower, whilst stearic acid, linolenic acid, and arachidic acid were slightly higher, in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 compared to its conventional counterpart. In the per-location statistical analysis, those differences observed in the across-location analysis and a number of additional statistically significant differences between maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart and commercial maize varieties occurred in*

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			Programme, Food and Agriculture Organisation: Rome.] [Lundry, D. R., [redacted], Miller, K. D. and Sorbet, R. (2007). Composition Analyses of Forage and Grain Collected from the Combined Trait Corn Product MON 89034 × TC1507 × MON 88017 × DAS-59122-7 Produced in the United States during the 2006 Field Season. Dossier EFSA/GMO/CZ/2008/62, Monsanto Company.]	*separate locations but not in all of them. For all parameters showing differences, the range of individual values of MON 89034 x 1507 x MON 88017 x 59122 was completely within the range of commercial maize varieties, except for three parameters (arachidic acid, vitamin B1, and folic acid), each of which showed a single sample in one location having a value slightly beyond this range, the average values of maize MON 89034 x 1507 x MON 88017 x 59122 were within the background range of literature and database values.*" "*The EFSA GMO Panel considered the observed compositional differences between grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial maize varieties, and concludes that forage and grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 do not differ compositionally from its conventional counterpart and are equivalent to commercial maize varieties, except for the newly introduced traits.*"
Austria	Federal Ministry of Health	D, 07.01 Comparative assessment	The notifier concludes, "The results of the compositional analyses have established the compositional equivalence of this maize and conventional maize grain, and as a consequence no further nutritional assessments of MON MON89034x1507xMON88017x59122 for use as or in feed are considered necessary." Anyhow, the assumption that nutritional adequacy can be assessed by chemical analyses still needs to be evidenced. Instead, it has been shown that the genetic modification can lead to discreet changes in the molecular structure of the transgenic protein (Prescott et al. 2005). Also, unexpected effects of certain genetically manipulation were described, which e.g. resulted in alterations of the peripheral and intestinal immune response of weaning	The EFSA GMO Panel notes that, in general terms, the outcomes of chemical analysis of key nutrients are commonly used to assess the nutritional value of diets, *e.g.*, for food and feed formulation. The references by Prescott and Finamore that the Member State refers to do not refer to MON 89034 x 1507 x MON 88017 x 59122 or its constituent single events. The safety of the single transgenic components has been previously evaluated for the dossiers on the single parental events, whilst the

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			and old mice (Finamore et al. 2008). Additionally, regarding the statistical analysis of the US and Canada field trials of GM maize 59122 (Buffington 2004) it may be added that, although, in general, the applied structure of the model is ok, the author used a linear mixed model to analyse the data but did not mention if the data follows a normal distribution or not; however, this is an assumption which is a prerequisite for using a linear mixed model. [Buffington, J. (2004). Agronomic Characteristics, Quantitative ELISA and Nutrient Composition Analysis of Hybrid Maize Line 59122 Containing the cry34Ab1, cry35Ab1, and pat Genes: United States and Canada Locations. Dossier EFSA-GMO-NL-2005-12, Pioneer Hi-Bred International, Inc.] [Finamore, A., Roselli, M., Britti, S., Monastra, G., Ambra, R., Turrini, A. and Mengheri, E. (2008). Intestinal and Peripheral Immune Response to MON810 Maize Ingestion in Weaning and Old Mice. J Agric Food Chem] [Prescott, V. E., Campbell, P. M., Moore, A., Mattes, J., Rothenberg, M. E., Foster, P. S., Higgins, T. J. and Hogan, S. P. (2005). Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity. J Agric Food Chem 53(23): 9023-9030.]	EFSA GMO Panel's opinion on MON 89034 x 1507 x MON 88017 x 59122 additionally considers any new information on the potential toxicity (including updated bioinformatics-supported comparisons of newly introduced proteins with toxins and allergens) and the potential for interaction among the single events combined in the stacked event. The data on the single event 59122 were evaluated previously (see EFSA opinion adopted on 23 March 2007; http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620768441.htm). Section 3.2.1 of that opinion notes that "The data from field trials performed in Europe were used by the GMO Panel as the primary source for the comparative assessment of the composition of maize 59122." These European data had been provided by the applicants in response to a request made by the EFSA GMO Panel. The opinion also notes that (section 3.2.2.) "*Compositional data were obtained by analysis of forage and kernels harvested from field trials performed in maize growing regions of Europe in 2003 and 2004. Statistical analysis of supplied data was performed on both individual and combined locations. The EFSA GMO Panel is of the opinion, that this set of compositional data is in compliance with the principles described in the Guidance document (EFSA, 2006a), and the selection of compounds follows the recommendations of OECD (2003).*"

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Austria	Federal Ministry of Health	D, 07.06 Effect of the production and processing	Pelleting process, silage process and distillation process (DDGS) were not or only scarcely mentioned. These processes should be described in sufficient detail to allow an assessment whether or not they are likely to modify the characteristics of the GMO product compared with its non-GM counterpart (EFSA 2006). No information about the fate of the DNA during those processes is provided. [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.]	In section 5.1.2 of the opinion, the EFSA GMO Panel notes that "*The scope of application EFSA-GMO-UK-2008-62 is for food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all derived products (e.g. starch, syrups, ethanol, maize oil, flakes, coarse and regular grits, coarse and dusted meal, flour, maize germ meal, maize gluten feed, condensed steep water, and maize gluten meal). The genetic modifications in maize MON 89034 x 1507 x MON 88017 x 59122 are intended to improve agronomic performance only and it is not intended to influence the nutritional properties, the processing characteristics, and overall use of maize as a crop.*"
Austria	Federal Ministry of Health	D, 07.08 Toxicology	First of all, we strongly criticise that the term 'margin of exposure', as used in the technical dossier (p. 106), is clearly misleading and contradicts all common European standards of an MOE-approach. So, it is stated by the notifier that MOEs were "calculated by dividing NOAELs from acute mouse gavage studies". This argument is inappropriate, as animal data for calculation of MOEs are usually derived from long-term carcinogenicity animal studies (EFSA/WHO 2006), and MOEs are used for risk assessment of substances that are both genotoxic and carcinogenic. Standards for carcinogenicity studies in rodents are normally life-term tests (EPA 1996; Barlow 2005; EFSA 2005). Furthermore, calculation of an MOE requires selection of an appropriate reference point from a dose-response curve, and, in addition to this, EFSA recommends that a benchmark dose (BMD) is used to obtain the MOE (EFSA 2005). All these facts are simply neglected by the notifier. Thus, the term 'MOE' which is definitely used incorrectly within the dossier should be eliminated. Moreover, all maize substances used as controls (grain 091, corn LH59xLH198 and one line simply named 'conventional parent') in the respective 90-days rat studies are poorly described as isogenic maize lines (Malley 2004; [redacted]; Kirkpatrick 2007). The notifier is, therefore, requested to submit detailed information on the genetic background and the origin of the control variety, as well as a breeding history in comparison to the	The EFSA GMO Panel thanks Austria for pointing towards this potential source of confusion. The data provided in the dossier can be briefly summarized as follows: Based on the expression levels of the newly expressed proteins measured during the field trials in the USA in 2006, and on human and animal consumption data for maize and derived products, the applicants estimated the potential intake of the newly expressed proteins by humans and animals consuming maize. Whilst the estimates were conservative, assuming a 100%-substitution scenario and no losses of newly expressed proteins during processing, the outcomes show that these levels were several orders of magnitude lower than the levels tested in the acute oral toxicity studies previously performed with these proteins without adverse effects. With regard to maize 59122, the applicants have clarified the pedigree of the comparator maize used in the various studies (including also the 90-days

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			test substance (GMO) used in the toxicological assessment. Concerning the statistical analysis it should be remarked that the two studies on MON88017 and MON89034, respectively (MacKenzie 2003; [redacted]; Kirkpatrick 2007), are longitudinal studies where measurements are taken over a period of 91 days each per week. These measurements are taken on the same animals, and so there should be some correlation between these measurements. So, it is not clear if the author took this dependency into consideration. Moreover, a classical ANOVA may not be the state of the art statistical method, especially if the response variabel does not follow a normal distribution. A classical ANOVA is maybe also not the state of the art statistical method regarding the rat study with GM maize 59122 (Malley 2004). In this study, the author transformed the response varable to obtain nomalised residuals. If using generalised linear models, this is possibly not necessary. The applicant analysed male and female separately; however, this can also be done in one analysis using adequate statistical models. [Barlow, S. (2005). Threshold of toxicological concern - A tool for assessing substances of unknown toxicity present at low levels in the diet. ILSI Europe] [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.] [EPA (1996). Proposed Guidelines for Carcinogen Risk Assessment. Fed Regist 61, 17960-18011.] [FAO/WHO (2001). Evaluation of Allergenicity of Genetically Modified Foods - Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology.] [[redacted] A 90-day feeding study in rats with MON88017 corn grain. Dossier EFSA-GMO-CZ-2005-27] [Kirkpatrick, J. B. (2007). A 90-day feeding study in rats with MON89034. Dossier EFSA-GMO-NL-2007-37][MacKenzie, S. A. (2003). Thirteen-Week Feeding Study with Transgenic Maize Grain (TC1507) in Rats. Dossier EFSA-GMO-RX-1507, E.I. du Pont de Nemours and Company.] [Malley, L. A. (2004). Thirteen-Week Feeding	study) in its reply to the EFSA GMO Panel on 22 March 2006 (for application 12). In addition, this information was provide for MON 89034 on 6 May 2008 (for application EFSA-GMO-NL-2007-37) The 90-days rat-feeding studies with maize containing the single parental events MON 89034, MON 88017, and 59122 have already been considered previously by the EFSA GMO Panel in its opinions on these single events. MON 89034: http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej909_maizeMON89034_en.pdf?ssbinary=true Maize 59122: http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_ov_op12_annexa_en,0.pdf?ssbinary=true Maize MON 88017: http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/gmo_op_ej1075_gm_maize_MON88017_en.pdf?ssbinary=true

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			Study with Transgenic Maize Grain (DAS-59122-7) in Rats. Dossier EFSA-GMO-NL-2005-12]	
Austria	Federal Ministry of Health	D, 07.08 Toxicology	In the technical dossier, the notifier says that the safety of all transproteins, Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT and CP4 EPSPS, expressed in the test material GM maize MON89034x1507xMON88017x59122 have been discussed in detail in other applications for authorisation. This concerns, amongst other things, history of safe use, structural description and digestion in simulated gastric fluid. In contrast to this, we would like to point out that: a) there is no history of safe use of the new recombinant protein expressed by an artificially arranged insert such as Cry1A.105. b) concerning all Bt toxins, a history of safe use cannot be argued on the basis of the safety of Bt sprays applied in organic farming. The inserted genes are truncated and arranged with expression modulating DNA parts originating from different organisms and permanently expressed compared to a tight timely Bt spraying schedule (Lewis et al. 1997; Sexton et al. 2007). c) the simulated gastric fluid is used at a pH of 1.2 only. FAO/WHO recommend using two pH conditions, pH 1.2 and pH 2.0 in order to cover a range of possible stomach conditions (FAO/WHO 2001). d) all eight transproteins used in acute toxicity tests (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, pat CP4 EPSPS) originated from microbial expression systems. Establishing structural and functional equivalence of this test proteins and the plant derived proteins adds uncertainties to the interpretation of the animal tests (Spök et al. 2008), thus, only limited information about the plant expressed transproteins can be obtained. Additionally, a 90-day rat feeding study with GM maize 59122 (Malley 2004) showed alterations of total protein and albumin levels, and we are still of the opinion that this study should be repeated, as recommended	The safety of the newly expressed proteins was previously evaluated by the EFSA GMO Panel in its opinions on the single parental events for this stacked event (MON 89034, 1507, MON 88017, 59122). Items considered for the safety of these proteins included *in vivo* toxicity testing with the purified protein (including 28-days study with the Cry34Ab1/Cry35Ab proteins that are also expressed in maize 59122, provided by the applicant at the EFSA GMO Panel's request, *in vitro* resistance to proteolytic degradation, bioinformatics-supported comparisons of the amino acid sequences of the newly expressed proteins with known toxins), and other characteristics of the proteins (e.g. glycosylation). The use of a bacterial analogue of a plant-expressed protein can be acceptable under certain conditions, as explained in section 7.8.1 of EFSA's guidance document: "*It is essential that the tested protein is equivalent to the newly expressed protein as it is expressed in the GM plant. If, due to the lack of sufficient amount of test materials (e.g. plant proteins), a protein is used which was produced by micro-organisms, the structural, biochemical and functional equivalence of the microbial substitute to the newly expressed plant protein must be demonstrated.*" With regard to the 90-days feeding study with maize 59122, the EFSA GMO Panel noted the following on the issue of clinical pathology in its previous opinion on maize 59122, section 4.2.4:

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			and remarked by Austria in the scientific comment on the triple stack GM maize 59122x1507xNK603 transferred to EFSA in September 2007. [FAO/WHO (2001). Evaluation of Allergenicity of Genetically Modified Foods - Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology.] [Lewis, W. J., van Lenteren, J. C., Phatak, S. C. and Tumlinson, J. H., 3rd (1997). A total system approach to sustainable pest management. Proc Natl Acad Sci U S A 94(23): 12243-12248.] [Malley, L. A. (2004). Thirteen-Week Feeding Study with Transgenic Maize Grain (DAS-59122-7) in Rats. Dossier EFSA-GMO-NL-2005-12, E.I. du Pont de Nemours and Company.] [Sexton, S. E., Lei, Z. and Zilberman, D. (2007). The Economics of Pesticides and Pest Control. International Review of Environmental and Resource Economics 1(3): 271-326.] [Spök, A., Dolezel, M., Eckerstorfer, M., Freigassner, M., Gaugitsch, H., Heissenberger, A., Karner, S., Klade, M., Proksch, M., Schneider, L., Treiber, F. and Uhl, M. (2008). Assessment of toxic and ecotoxic proerties of novel proteins in GMOs. BMGFJ. Vienna.]	"*According to the original study report, no adverse diet-related differences were observed with respect to clinical signs of toxicity, ophthalmological observations and neurobehavioral assessments, clinical pathology, organ weights and gross or microscopic findings in rats receiving the maize 59122 diet compared with the four combined control groups. In addition, there were no adverse, diet-related differences in mean body weight, body weight gain, food consumption or food efficiency. However the EFSA GMO Panel did not consider the statistical analysis as adequate, because the comparisons were made between groups fed maize 59122 and the four combined control groups. Therefore a new statistical analysis was requested. In addition, information regarding the origin of the non-GM control maize with comparable genetic background was requested. The new statistical analysis revealed no significant differences in final body weight, body weight gain, food consumption and food efficiency between rats fed the maize 59122 diet compared with the non-GM control maize. In the clinical pathology examinations, male rats receiving the maize 59122 diet showed statistically significant decreases in absolute reticulocyte count and red cell distribution width as well as increases in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Females showed an increase in platelet count. These differences were small, and the values were generally comparable with those of other control groups in this study and/or fell within the ranges for the historical control means for rats of the same strain in other subchronic feeding studies. In addition, there were no statistically significant differences in other parameters which are expected*

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				to be affected in case of relevant effects. The EFSA GMO Panel therefore does not consider the observed differences as toxicologically relevant."
Austria	Federal Ministry of Health	D, 07.08 Toxicology	Furthermore, according to EFSA, a potential for increased toxicity and/or allergenicity to humans and animals or for modified nutritional value due to the stacked events may arise from additive, synergistic or antagonistic effects of the gene products or by these produced metabolites (EFSA 2007). But the safety of all newly expressed proteins in animal models applied simultaneously and combined was not assessed in the dossier. Insecticidal Cry proteins produced by GM plants as well as transproteins conferring tolerance to herbicides constitute a sum of new plant constituents possibly interacting within the organism. So far, there is absolutely no scientific knowledge about such new combinations and possibly resulting additive and/or synergistic effects. Therefore, at least one subchronic feeding study (90-days) with rodents with the whole GM maize plant (MON89034x1507xMON88017x59122) should be carried out. Additionally, the introduction of multigeneration studies focussing on reproduction in the risk assessment process should be considered, at least on a case-by-case basis. So far, although GM crops have now been grown for over 20 years, only very few life-term and/or multigeneration studies have been carried out (Domingo 2007; Dona and Arvanitoyannis 2009). Moreover, it is suggested to carry out mutagenicity tests on bacteria with the transproteins. [Domingo, J. L. (2007). Toxicity studies of genetically modified plants: a review of the published literature. Crit Rev Food Sci Nutr 47(8): 721-733.] [Dona, A. and Arvanitoyannis, I. S. (2009). Health risks of genetically modified foods. Crit Rev Food Sci Nutr 49(2): 164-175.] [EFSA (2007). Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. The EFSA	At the request of the EFSA GMO Panel the applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.1, that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.*" According to the approach outlined by the EFSA Guidance Document and the *Codex alimentarius* guidelines (to which also Austria has subscribed), animal safety tests and other tests with GM plant-derived foods are not required *per se* but on a case-by-case basis, based on indications, for example, of certain unintended effects or substantially modified composition. Given the EFSA GMO Panel's conclusion that interactions that might impact on safety are unlikely, there is no need to carry out such studies

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Austria	Federal Ministry of Health	D, 07.09 Allergenicity	In the technical dossier, the notifier stresses that a protein is not likely to be an allergen if the protein represents only a very small portion of the total protein in the grain. Anyhow, even though low concentration of introduced proteins in tissues, that may be consumed, and the rapid digestibility in simulated digestive fluids might provide additional safety, it should not be neglected that minimal traces of substances can trigger allergic reactions (Madsen et al. 2009). Furthermore, in the dossier it is remarked, "the history of safe use of the Cry proteins by humans on agricultural crops for over 10 years, either as the active ingredients in Bt microbial pesticides and/or in biotechnology derived food and feed crops (maize and cotton). There are no known reports of allergy or toxicity to Bt or to the Cry proteins" (p. 83). Actually, the simple fact that GM corn has been grown for over 10 years on millions of hectars, and that no reports about adverse effects have been transmitted is no proof for safety. The same could have been said about DDT and many other synthetic agricultural supplies that are now banned. Since GM products have not been labelled in the USA and Canada, no epidemiological survey of potential effects has been conducted. Thus, if the GM food may or may not play its part in the increase of nutrition-related health distubances such as allergies and food intolerances cannot be clarified. Anyway, allergic reactions against Bt toxins have been reported in farm workers exposed to Bt containing pesticides (Bernstein et al. 1999) [Bernstein, I. L., Bernstein, J. A., Miller, M., Tierzieva, S., Bernstein, D. I., Lummus, Z., Selgrade, M. K., Doerfler, D. L. and Seligy, V. L. (1999). Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environ Health Perspect 107(7): 575-582.] [Madsen, C. B., Hattersley, S., Buck, J., Gendel, S. M., Houben, G. F., Hourihane, J. O., Mackie, A., Mills, E. N., Norhede, P., Taylor, S. L. and Crevel, R. W. (2009). Approaches to risk assessment in food allergy: report from a workshop "developing a framework for assessing the risk from allergenic foods". Food Chem Toxicol 47(2): 480-489.]	The allergenicity of the newly expressed proteins has been assessed according to the weight-of-evidence approach devised by the EFSA Guidance Document and *Codex alimentarius* guidelines (to which Austria also subscribes), as evaluated by the EFSA GMO Panel in its opinions on the single events. This weight-of-evidence approach includes, for example, a consideration of the history of allergenicity of the source and recipient of the transgene, bioinformatics-supported comparisons of the amino acid sequence of the newly expressed protein with the sequences of known allergens, and resistance of the newly expressed protein to *in vitro* proteolysis. The quoted publication by Bernstein (1999) concludes that, among others, "*it is unlikely that consumers would develop allergic sensitivity after oral exposure to transgenic foods (e.g., tomatoes, potatoes) that currently contain the gene encoding this protein.*"

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Austria	Federal Ministry of Health	D, 07.10 Nutritional assessment of GM food/feed	A broiler study ([redacted]) is introduced as a confirmatory feeding with 9 treatments and 900 broiler chickens. As the statistical analyses were conducted by the applicant (Monsanto Statistics Technology Center), the study cannot be considered wholly independent. For instance, according to the European Regulation 428/2008 (assessment and authorisation of feed additives - annex II, section II, 2.6.1.3.), "Performance characteristics of in-house validated methods shall be verified by testing the method in a second, accredited and independent laboratory". This, and other, minimal standards, that are mentioned within the Regulation 428/2008, and that are regarded essential for the charcteristics of submitted studies, should also be applied for GMOs. Nine treatments with 100 birds each were investigated, but only the data of 8 treatments or 800 birds are shown in the tables. (1 test, 1 control and six reference groups). What was the ninth treatment (later named treatment 6), which was later named treatment 6, and was not described or defined, used for? We would also like to remark that only 2 of the 9 treatments were directly relevant for the safety of the GMO, the treatment with the GM test corn and that with the close-genetic variant. In other words, only 22% of the birds were fed either with the GM corn or the control. Analyses of pesticides, mycotoxins, amino acids, fatty acids, nutrients and anti-nurients as well as the verification of presence and absence of the test, control and reference corn were performed on the grain only and not on the complete feed diet. This is not in line with current guidance, which requires description of manufacture and quantitative composition of the diet (EFSA 2008). Furthermore, the soybeans provided by Global Poultry Consulting Inc. and used for the diet formulation were not tested for potential GM contamination. Also, analyses of heavy metals (cadmium, mercury, arsenic, lead) and vitamins were not conducted at all.	The pertinent paragraph in the quoted regulation pertains to the validation of analytical methods for feed additives. It is noted that analytical methods for detection GMOs are also validated by JRC, which is outside the remit of the EFSA GMO Panel's mandate. There is currently no legal requirement for applicants to outsource their research on GMOs. Both issues do not specifically pertain to the application on MON 89034 x 1507 x MON 88017 x 59122. In the evaluation of the chicken broiler study data, the EFSA GMO Panel's focus was on the comparison between test and control maize-fed groups. The following extract from section 5.1.6 of the opinion summarizes this as follows: "*A 42-day broiler chicken feeding study with adjusted diets containing grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 was evaluated in the frame of the current marketing application1. Both male and female chicken received adjusted diets containing 61-64% of one of eight maize lines, i.e. grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart (XE6001), and six commercial maize varieties.* " *"No statistically significant differences were observed between the group fed adjusted diets containing grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart, except for a higher absolute and relative (%) fat pad weight in the group fed GM-maize as compared to that fed control maize (47 v. 43 g; 1.9 v. 1.7% of live weight). However, these differences were not observed in the comparison*

[1] Technical Dossier/ Section D7.10

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			It is also stated that "the feed was pelleted through a 5-mm die with live steam addition, and starter diets were fed as crumbles and grower/finisher diets were fed as pellets". The application of heat and pressure could inactivate proteins (Spök et al. 2008); therefore, in feeding tests with the aim to investigate the potential effect of recombinant proteins this procedure is not recommended. On page 16 (C. Assays), it is mentioned that diets were assayed for analytes in complete diet by the University of Missouri. However on page 41 (appendix II, table 2) and pages 42, 43 (appendix II table 3) the term "Calculated Nutrient Composition" was used. [redacted]). Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7, Control, or Reference Corn. Dossier EFSA/GMO/CZ/2008/62, [redacted] [EFSA (2008). Technical guidance on tolerance and efficacy studies in target animals - Prepared by the Panel on Additives and Products or Substances used in Animal Feed. The EFSA Journal 778: 1-14.] [Spök, A., Dolezel, M., Eckerstorfer, M., Freigassner, M., Gaugitsch, H., Heissenberger, A., Karner, S., Klade, M., Proksch, M., Schneider, L., Treiber, F. and Uhl, M. (2008). Assessment of toxic and ecotoxic proerties of novel proteins in GMOs. BMGFJ. Vienna.]	*between the group fed GM-maize and each of the groups fed commercial maize varieties. The observed differences in fat pad weights were also observed in female chicken fed with GM maize compared with non-GM maize when analyzed in a by-gender statistical analysis. In the absence of any other treatment-related effects on performance, the EFSA GMO Panel does not consider the statistically significant difference in fat pad weights to be of biological relevance. The broiler chicken feeding study supported the results of the comparative compositional analysis and confirmed that grains produced by maize MON 89034 x 1507 x MON 88017 x 59122 are nutritionally equivalent to grains of the conventional counterpart and six commercial maize varieties."* It should be remarked here that this chicken feeding study is not regard by the EFSA GMO Panel as a toxicity study for the newly expressed proteins. Moreover, pelleting is a commonly used process for preparing animal feeds.
Austria	Federal Ministry of Health	D, 07.10 Nutritional assessment of GM food/feed	Moreover, it should be remarked that the results of feed intake, which was determined twice during the whole feeding period on day 21 and day 42, showed a number of significant differences between the treatments, but the GM test corn with the overall lowest feed intake was not significantly different from the control. Thus, the feed intake of Golden Harvest 9166 and Dekalb DKC61-50 for instance was significantly higher than of MON89034x1507xMON88017x59122. The same is true for the feed conversion ratio. It seems to be surprising that some reference corn variants were accepted much more readily than others, keeping in mind that the diet preparation was comparable. Also, significant differences in bird weight on day 42 were recorded. Not between the GM corn and its control, but the mean for MON89034x1507xMON88017x59122 was significantly lower than the	As noted above, the primary focus of the evaluation of the chicken study data in the EFSA GMO Panel's opinion on maize MON 89034 x 1507 x MON 88017 x 59122 is on the comparison between the test maize and its non-GM control (not the reference lines), in line with EFSA and *Codex alimentarius* guidance. If differences are observed in this comparison, the reference groups can then provide further insight into the background variation for the specific parameter showing this difference. With regard to data on males and females separately, the EFSA GMO Panel requested and received from the applicants additional data with a

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			mean for Golden Harvest H9166. Another point is that no separate information on the weight development of males and females is given. Furthermore, the feed intake was determined as the amount consumed per pen. This calculation is not on an individual basis and might mask differences within individuals by just investigating the group average. In this regard, we would like to point to the fact that protocols must be sufficiently sensitive to detect any effects at the lowest recommended dose (EFSA 2008). What can be concluded is, however, that although, the GM corn, using the mixed model, showed no significantly different impact on broiler performance, other corn varieties enhanced feed intake and weight development significantly better than the GM corn variant did. [EFSA (2008). Technical guidance on tolerance and efficacy studies in target animals - Prepared by the Panel on Additives and Products or Substances used in Animal Feed. The EFSA Journal 778: 1-14.]	by-gender (male or female) statistical analysis of the outcomes of the study. The point on the feed intake per pen is taken, whilst it is noted that it is common practice to measure feed intake per pen, which has previously also been done in the chicken feeding studies for other dossier (the replication is then the number of pens).
Austria	Federal Ministry of Health	D, 07.10 Nutritional assessment of GM food/feed	Statistical analysis ([redacted]): In the two models different numbers of treatments were analysed. In model 1, data from nine treatments were analysed, of which one treatment (no. 6) was never introduced in the study. Model 1 had two factors, diet and sex. If the interaction of diet and sex was not significant ($p \geq 0.15$), diet comparison was not done for each sex separately. If the interaction was significant ($p < 0.15$), diet comparison for each sex was performed. Why is the level of significance here 0.15 and not 0.05? And, why was no gender specific analysis performed? Model 2 used data from eight treatments only; the analysis compared the test group with a sample of the population of the control and the reference groups. No gender analysis was performed, unless there was a significant diet-by-sex interaction. No separate comparison of the test group and the control group and no gender analysis were performed. This statistical analysis has to be considered insufficient and the	A gender-specific analysis was requested from – and provided by – the applicants to the EFSA GMO Panel. The only statistically significant differences thus observed between test and control was for fat pad weights in female animals (same as for the overall comparison combining both genders). This is summarized as follows in section 5.1.6 of the opinion*: "No statistically significant differences were observed between the group fed adjusted diets containing grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart, except for a higher absolute and relative (%) fat pad weight in the group fed GM-maize as compared to that fed control maize (47 v. 43 g; 1.9 v. 1.7% of live weight). However, these differences were not observed in the comparison between the group fed GM-maize and each of the*

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			applicant is requested to provide a new statistical analysis or a scientific rationale on the discrepancies mentioned above. [redacted]. Comparison of Broiler Performance and Carcass Parameters When Fed Diets Containing MON 89034 × TC1507 × MON 88017 × DAS-59122-7, Control, or Reference Corn. Dossier EFSA/GMO/CZ/2008/62, [redacted]	*groups fed commercial maize varieties. The observed differences in fat pad weights were also observed in female chicken fed with GM maize compared with non-GM maize when analyzed in a by-gender statistical analysis. In the absence of any other treatment-related effects on performance, the EFSA GMO Panel does not consider the statistically significant difference in fat pad weights to be of biological relevance.* "
Austria	Federal Ministry of Health	D, 10 Potential changes in the interactions of the GM plant with the biotic	D.9 Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification: The notifier concludes that due to containment measures during the proposed use of GM maize MON88017xMON89034x1507x59122 only limited exposure of the environment is to be expected. Only accidental spillage of viable maize grains is considered in the ERA presented by the notifier. Thus, the notifier does not adequately address other exposure routes of products derived from GM maize MON88017xMON89034x1507x59122 and of transgenic constituents of this GM maize hybrid. Additional exposure may, for instance, result from feed use (leading for instance to exposure to non-target organisms especially in the soil via organic fertiliser) or from waste materials and sewage from the feed industry (which may lead to the exposure of non-target organisms in aquatic eco-systems (Rosi-Marshall et al. 2007)). A number of studies indicate the presence of immunoreactive parts of cry-proteins in the faeces of ruminants fed GM-feed (Einspanier et al. 2004; Lutz et al. 2006) and the possibility for sustained presence of these cry-toxins in soil material (Lee et al. 2003; Bayerische Landesanstalt für Landwirtschaft 2005). Actual survival of GM maize resulting from accidental spillage could remain undiscovered if not covered by an effective monitoring strategy (see below). Outgrowth of such maize grains would result in exposure to non-target organisms in European environments, a szenario which is not considered in this notification by the notifier. Therefore, the notifier	The scope of the application includes food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of these the individual events as present in its segregating progeny~~,~~ and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny) during transportation and processing. 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 89034 x 1507 x MON 88017 x 59122 during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize

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			is requested to present a more comprehensive exposure assessment. [Bayerische Landesanstalt für Landwirtschaft (2005). Monitoring der Umweltwirkungen des Bt-Gens. Schriftenreihe 7.] [Einspanier, R., Lutz, B. and Rief, B. (2004). Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. Eur Food Res Technol 218: S269–273.] [Lee, L., Saxena, D. and Stotzky, G. (2003). Activity of Free and Clay-Bound Insecticidal Proteins from Bacillus thuringiensis subsp. israelensis against the Mosquito Culex pipiens. Appl. Environ. Microbiol. 69(7): 4111-4115.] [Lutz, B., Wiedemann, S. and Albrecht, C. (2006). Degradation of transgenic Cry1Ab DNA and protein in Bt-176 maize during the ensiling process. J Anim Physiol Anim Nutr (Berl) 90(3-4): 116-123.] [Rosi-Marshall, E. J., Tank, J. L., Royer, T. V., Whiles, M. R., Evans-White, M., Chambers, C., Griffiths, N. A., Pokelsek, J. and Stephen, M. L. (2007). Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proc Natl Acad Sci U S A 104(41): 16204-16208.]	MON 89034 x 1507 x MON 88017 x 59122 grains or through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts.
Austria	Federal Ministry of Health	D, 10 Potential changes in the interactions of the GM plant with the biotic	D.9.5 Interactions of the GM plant with non-target organisms: The notifier identifies as target organisms "certain Lepidopteran and Coleopteran pest species" without including data on the actual range of species which may be affected by the number of Bt-toxins which are present in GM maize MON88017xMON89034x1507x59122. Specifically, no data on efficacy conducted with the whole GMP – neither in the laboratory nor in the field - are provided by the notifier. Therefore, the possibility of trait interaction (e.g. synergistic effects) resulting from the simultaneous expression of several Bt-toxins is not accounted for. In addition, it is known that the toxicity and consequently the specificity of a Cry-protein is dependent on its structure and the conditions of ingestion, and thus, can be influenced by various toxin-related, organism-related and environmental factors (Spök et al. 2008). Therefore, it is necessary to complement laboratory studies conducted	The scope of the application includes food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of these the individual events as present in its segregating progeny~~,~~ and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny) during transportation and processing. There are no indications of an increased likelihood of establishment and spread of feral maize plants in

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			with purified proteins administered in an optimised artificial diet with studies conducted to address realistic exposure scenarios. The notifier is, thus, requested to submit a more realistic exposure assessment of effects on non-target organisms. [Spök, A., Dolezel, M., Eckerstorfer, M., Freigassner, M., Gaugitsch, H., Heissenberger, A., Karner, S., Klade, M., Proksch, M., Schneider, L., Treiber, F. and Uhl, M. (2008). Assessment of toxic and ecotoxic proerties of novel proteins in GMOs. BMGFJ. Vienna.]	case of accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize MON 89034 x 1507 x MON 88017 x 59122 grains or through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts.
Austria	Federal Ministry of Health	D, 12.02 Case-specific GM plant monitoring	D.11.3 Case-specific GM plant monitoring: The applicant concludes that based on the submitted risk assessment the adverse effects on humans and animals are negligible, and thus, case-specific monitoring is not appropriate. However, this conclusion needs to be justified by more data addressing the concerns with regard to the presented risk assessment. Specifically, the possibility of synergistic effects of the transgenic proteins present in GM maize MON88017xMON89034x1507x59122 should be addressed in an improved way. The notifier additionally indicates that environmental exposure of GM maize MON88017xMON89034x1507x59122 may occur by unintended release of the GM maize e.g. via losses during loading or unloading of maize (technical dossier, p. 147). However, the notifier does not propose to establish surveillance or management systems which are suitable to monitor and detect possible unintended environmental exposure by accidental spillage or release of GM maize MON88017xMON89034x1507x59122, as well as other routes of exposure of the environment to (waste) materials from processing or	No event-related effects on human and animal health have previously been identified in the EFSA GMO Panel's opinions on the single parental events. At the request of the EFSA GMO Panel. The applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.1, that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.*"

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			use taking into account the many transgenic constituents contained in GM maize MON88017xMON89034x1507x59122. An active monitoring of small size grain losses at diverse locations including an analysis of potential areas of concern and exposure pathways should be performed. Thus, in order to cover the risk of accidental spillage or unintended release into the environment of GM maize MON88017xMON89034x1507x59122 a case-specific monitoring plan should be proposed. This comprises the monitoring along transportation routes, ports and harbours, processing plants, etc. Furthermore, potential synergistic effects of the transgenic constituents should be covered by a specific monitoring of animal health.	In addition, in section 5.1.7, the EFSA GMO Panel concludes that "*No biologically relevant compositional, agronomic and phenotypic changes were identified in maize MON 89034 x 1507 x MON 88017 x 59122 when compared with its conventional counterpart and commercial maize varieties. Furthermore, the overall intake or exposure is not expected to change because of the introduction of maize MON 89034 x 1507 x MON 88017 x 59122 into the market. The EFSA GMO Panel therefore considers maize MON 89034 x 1507 x MON 88017 x 59122 to be as safe as its conventional counterpart and that post-market monitoring (EFSA, 2006) of the food/feed derived from maize MON 89034 x 1507 x MON 88017 x 59122 is not necessary.*" The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on post-market environment monitoring (PMEM) (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b):

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				Details of the specific plans and methods of monitoring in each country should not be included in the original application. The GMO Panel advises that the application should describe the general approaches and methods that the applicant would apply in different commercialisation sites, including the type of dialogue that would be established with risk managers in each Member State. (…) Thus detailed local arrangements will be developed by the applicant after the application has been accepted (…). See section 6.1.3 of the scientific opinion.
Austria	Federal Ministry of Health	D, 12.03 General Surveillance of the impact of the GM plant	D.11.4 General surveillance for unanticipated adverse effects: The general surveillance plan proposed by the notifier is limited to providing information to third parties involved in the monitoring and to collecting information via key networks. It remains unclear which specific institutions will be informed and participate in monitoring. The notifier, thus, should give an overview on the national organisations involved in each individual EU member state and supply information to which degree these institutions will be involved. The notifier shall document the commitment of the organisations which will be part of the monitoring network to actively take part in the monitoring and to assist the notifier in the monitoring. The monitoring plan shall describe the responsibilities of members to the monitoring network and better specify the responsibilities of the notifier for collecting and analysing information. Moreover, it is unclear why no veterinary or medical associations are included in the proposed surveillance. GM maize MON88017xMON89034x1507x59122 will be used for animal feed, and therefore, identification of occurrence of adverse effects of the GMO on animal health need to be included in the general surveillance. The methodology of the proposed general surveillance is based on	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicants in the general surveillance plan. Please note that the EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.

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			passively collecting information. A more active approach of GS, which includes specific activities for monitoring accidental spillage, should also be employed by the notifier (see CSM). The notifier refers to the routine surveillance based on the HACCP principles (annex 1). Since the description provided is very general, the notifier shall outline how these principles match with the requirements of an environmental monitoring plan of GM maize MON88017xMON89034x1507x59122. In summary, the plan provided by the notifer is too general and too imprecise for a sufficient surveillance of unintended effects on human or animal health and the environment. Thus, the monitoring plan should be revised.	
BELGIUM	Belgian Biosafety Advisory Council	General comments	Serious problems are not really expected by the application of MON89034 x 1507 x MON88017 x 59122 maize. The safety aspects of the multiple challenge, due to the combination of the newly inserted proteins, are rather weakly demonstrated.	No event-related effects on human and animal health have previously been identified in the EFSA GMO Panel's opinions on the single parental events. At the request of the EFSA GMO Panel, the applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.1, that "*Determination of the levels of the newly expressed proteins in grain harvested from maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (MON 89034, 1507, MON 88017, 59122) (see section 3.1.4). Based on the known functions and modes of action of the newly expressed proteins, the EFSA GMO Panel considers the occurrence of interaction of these proteins (Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34/35Ab1, CP4 EPSPS, and PAT) unlikely.*"
BELGIUM	Belgian Biosafety Advisory	A. General information	It is important to note that the safety of MON 89034 and MON 88017 is still under scientific review by the EFSA GMO panel (Technical dossier I, page 14).	The single events 1507, 59122, MON 88017 and MON 89034 have been the subjects of previous assessments and have received an EFSA opinion in

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	Council			favour of their authorisation (EFSA, 2004, 2005a, b, 2007b, 2008, 2009a, b).
BELGIUM	Belgian Biosafety Advisory Council	C. Information relating to the genetic modification	P31 of the Technical dossier: could the authors state why this construct also contains –what it seems- partial cry1F and pat fragments? What is the size is of these fragments and why is hybridisation not seen in Southern blots?	The presence of partial *cry1F* and *pat* fragments in 1507 is a result of the particle acceleration method used to transform maize with the PHI8999 fragment. A detailed description of the insert was present in the single event dossier. The Southern and sequencing data have shown the presence of the partial fragments.
BELGIUM	Belgian Biosafety Advisory Council	D, 02 Information on the sequences actually inserted or deleted	P36 of Technical dossier: is it possible to add data on the chromosome location of the four inserts (and segregation analysis in relation with the breeding scheme in fig 13 p51)? P39 of Technical dossier: what can be said about the band of 4.2 Kb? Is this due to partial restriction of genomic DNA?	Information on the chromosomal location of the insert(s) is not considered necessary to carry out the risk assessment. The band of 4.2 kb observed in 1507 and in the stacked line is a result of hybridisation of the partial *cry1F* fragment with the Cry1F probe.
BELGIUM	Belgian Biosafety Advisory Council	D, 03 Information on the expression of the insert	In part 1 of the technical dossier we can read p.45 “For the PAT protein, expression was higher in the combined trait product as compared to 1507 and 59122” but the Table 12 (PAT) shows that the values for PAT protein levels in grain collected from MON 89034 × 1507 × MON 88017 × 59122 (0.050 µg/g dw) are similar to those of 59122 (0.049). Is there no contradiction between the statement “… the levels of Cry1A.105….are comparable to the protein levels in the positive controls…” (Technical dossier, part I, page 45) and the data provided in Table 6 for this protein (4.3 vs 2.8 in the control)? (almost no overlap in range; means are about 3 SD different). P45 we are not sure whether statistically there is a difference in Pat levels between 59122 and (89034 x 1507 x 88017 x 59122) as stated and therefore to our opinion the sentence: "This is likely due to the presence of multiple copies of the pat gene…" should be deleted.	The EFSA GMO Panel takes note of this comment. Considering the scope of the application and the safety of the newly expressed proteins, the values reported can be considered “comparable” (whether statistically significantly different or not). It should be noted that differences in expression levels of newly expressed proteins between stacked lines and the single events are not uncommon.

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BELGIUM	Belgian Biosafety Advisory Council	D, 07.01 Comparative assessment	- Table 21, Technical dossier, part I, page 73. Is there no mistake in the reported mean value for linoleic acid in the control grain? This value seems unlikely to me (for a non high-oleic acid corn oil). - The presence of trypsin and chymotrypsin inhibitors have been described in corn (Shulima et al., 1985). No data are reported on the level of these antinutrients. - Palmitoleic acid is not mentioned in table 20 of the Technical Dossier, part I, page 69. Yet it is known to be a minor component in corn oil and was found in some positive controls. The claim N/A (not available) is unlikely as the fatty acid composition was determined by gas chromatography. This omission is of no nutritional importance however. - It is not clear what is meant with "maize tissues" in the second paragraph on page 65 of Technical Dossier, part I. (…maize tissues that are consumed??) Printing error? Shulmina AI, Dronova LA, Shubin VV, et al. (1985) Determination of the secondary structure of the chymotrypsin inhibitor from corn by the circular-dichroism method. BIOCHEMISTRY-MOSCOW 50, 7: 980-982	The EFSA GMO Panel thanks Belgium for its detailed observations. The value in Table 21 indeed does not appear to match the data reported in Lundry (2007) for the values observed in location IA-2 (both test and control are more similar, reported as % dw; see PDF pages 63 and 75). The tables with detailed outcomes in Lundry (2007) show that palmitoleic acid was actually measured. The bioavailability of phosphorus treated on page 65 of the technical dossier indeed relates to the bioavailability from the consumed plant. With regard to trypsin inhibitor as antinutrient of maize kernels, it appears that this has indeed been described in scientific literature, However, table 12 of the OECD consensus document on maize (2002*) with recommended does not include the trypsin inhibitor. In the section on antintutrients, the OECD document notes that "*Maize contains low levels of trypsin and chymotrypsin inhibitors, neither of which is considered nutritionally significant (White and Pollak, 1995).*" *http://www.olis.oecd.org/olis/2002doc.nsf/LinkTo/NT00002F66/$FILE/JT00130429.PDF
BELGIUM	Belgian Biosafety Advisory Council	D, 07.03 Selection of compounds for analysis	Comment 1 The selection of the compounds for analysis is according to the OECD consensus document 6 (2002). However, considering that DIMBOA and its glycoside may total 1% of dry weight in conventional corn plants (Klun et al.,1969) and that mutagenic effects in human cell lines have recently been demonstrated (Buchmann et al., 2007), it seems of value to determine these components in corn containing stacked events. Hormonal effects of its degradation product MBOA have been described in rodents (in OECD report 6, page 28). It is claimed that 2-furaldehyde was determined (Technical Dossier I,	With regard to the levels of DIMBOA and MBOA, OECD concludes that "Analysis of DIMBOA in maize silage is not recommended (OECD, 2002) because of the high variability of its levels among maize varieties, and the fragmentary knowledge on its toxicology." Lundry (2007) provides the outcomes for 2-furaldehyde (= furfural) analysis, showing that levels were below limits. Issues regarding definition of carbohydrates and fiber are not specific for this dossier and therefore

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			page 82). Yet, I have found no data on levels of this component. Did I miss them or were they not reported? Although furfural has GRAS status, it has been suggested that an increase of the furfural level in food stuff should be avoided and that furfural is considered as a dietary risk factor for cancer (Feron et al., 1991). Comment 2 Grain and forage are analyzed for nutrients, according to the OECD documents. In addition other constituents with growing importance are included in the study. On the other hand a rather traditional approach was chosen for the proximate nutrients. No information is available on the composition of the carbohydrate fraction as it is calculated “by difference”. This is regrettable from a nutritional point of view as more and more attention is given to the type of carbohydrates present in human food. The same remark further applies for the fibre fraction, as mentioned several times before in previous dossiers. The OECD document needs to be adapted to current knowledge in human nutrition (see also EU definition of fibre). Buchmann CA, Nersesyan A, Kopp B, et al. (2007) Dihydroxi-7-methoxy-1,4-bezoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), two naturally occurring benzoxazinones contained in sprouts of Gramineae are potent aneugens in human-derived liver cells (HepG2). Cancer Letters 246: 290-299. FeronVJ, Til HP, Devrijer F et al. (1991) Aldehydes- occurence, carcinogenic potential mechanisms of action and risk assesment. Mut Res 259:363-385. Klun JA, Robinson JF (1969) Concentration of two 1,4 benzoxazinones in dent corn of various stages of development of the plant and its relation to resistance of the host plant to the European corn borer J Econ Entomol 62: 214-220.	could the topic of a more general discussion within other international consensus-building consortia.

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BELGIUM	Belgian Biosafety Advisory Council	D, 07.08 Toxicology	Comment 1 In case of MON 89034 × 1507 × MON 88017 × 59122 there may be a multiple challenge, which can be more harmful than any individual newly inserted proteins. It is highly desirable to refer to studies that have demonstrated that the combination of all these newly inserted proteins is not detrimental. However, the modes and sites of biological activity are different for the Cry, PAT and CP4 EPSPS proteins and there is no known or conceivable mechanism of interaction between these proteins which could lead to adverse health effects in animals or humans. Does this observation really guarantee full safety ? Comment 2 A study of Séralini et al. (2007) revealed signs of hepatorenal toxicity in rats due to the genetically modified Maize MON 863, which contains a variant of the Bacillus thuringiensis Cry3Bb1 gene. MON 89034 × 1507 × MON 88017 × 59122 produces Cry3Bb1 insecticidal protein and may therefore be similar to MON 863. Comment 3 According to the Ouellet et al. (2003) the TMR contained 0.446 maize silage (containing approximately 70 g protein/kg dry matter; DM) and 0.188 cracked maize (containing approximately 100 g protein/kg DM), resulting in 40 g maize protein/kg dietary DM. However, DDGS (containing approximately 300 g protein/kg DM), the by-product from the bioethanol production is sometimes incorporated in diets for dairy cows at 0.3 - 0.4 (Hippen et al, 2003; Kalscheur et al., 2004; Janicek et al., 2008), and distillers grains were be used at 50% in diets for finishing beef cattle (Roeber et al., 2005). Diets with 40% DDGS may yield ±120 g maize protein/kg dietary DM, which means that: - the protein amount coming from maize, via DDGS, is 3 x higher than the protein amount coming from directly from MON 89034 × 1507 × MON 88017 × 59122 grain and/or silage - this protein also contain about 3 x more newly inserted protein	To comment 1: No event-related effects on human and animal health have previously been identified in the EFSA GMO Panel's opinions on the single parental events. At the request of the EFSA GMO Panel, the applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section5.1.4.1, that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.*" To comment 2: The EFSA GMO Panel has previously considered the data reported by Seralini (see report dated 28 June 2007) and concluded that this re-analysis of the 90-days study has raised no new concerns (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178621165358.htm) To comment 3: Thanks for this interesting insight into potentially higher levels of exposure to protein derived from maize MON 89034 x 1507 x MON 88017 x 59122 if dried distillers' grain were to be used. Whilst this conceivably will affect the estimated intake, this would still yield low estimated intakes. The issue is addressed in the dossier,

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			What is the effect of such a diet on the safety for the animals? However, the animal feeding study, reported by [redacted] and conducted with relatively high incorporation levels in the diets of broilers, did not show health problems which can be attributed to this MON 89034 × 1507 × MON 88017 × 59122 maize, which is an indication of a safe use in monogastric animals. Comment 4 Has an up-to-date sequence homology search been performed for each of the proteins? Hippen, A.R., Linke, K.N., Kalscheur, K.F., Schingoethe, D.J., Garcia, A.D. 2003. Increased concentration of wet corn distillers grains in dairy cow diets. J. Dairy Sci. 86 (Suppl. 1): 340 (Abstr.). Janicek, B.N., Kononoff P.J.,, Gehman, A.M., Doane, P.H. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. J. Dairy Sci. 91: 3544-3553. Kalscheur, K.L., A.L. Justin, A.L. Hippen, and D.J. Schingoethe. 2004. Increasing wet distillers grains in the diets of dairy cows on milk production and nutrient utilization. J. Dairy Sci. 87 (Supp. 1): 465-466. (Abstr.). Ouellet, D.R., Lapierre, H., Chiquette, J. 2003. Effects of corn silage processing and amino acid supplementation on the Performance of lactating dairy cows. J. Dairy Sci. 86: 3675-3684 Roeber, D.L., Gill, R.K., DiCostanzo, A. 2005. Meat quality responses to feeding distiller's grains to finishing Holstein steers. J. Anim. Sci. 83: 2455–2460. Séralini, G.E., Cellier, D., Spiroux de Vendomois, J. 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch. Environ. Contam. Toxicol. 52: 596-602.	which can be summarized as follows, in brief: Based on the expression levels of the newly expressed proteins measured during the field trials in the USA in 2006, and on human and animal consumption data for maize and derived products, the applicants estimated the potential intake of the newly expressed proteins by humans and animals consuming maize. Whilst the estimates were conservative, assuming a 100%-substitution scenario and no losses of newly expressed proteins during processing, the outcomes show that these levels were several orders of magnitude below the levels having no adverse effects in the acute oral toxicity studies previously performed with these proteins. The issue of high-inclusion rates of protein-rich feeds is a general one and not specifically related to MON 89034 x 1507 x MON 88017 x 59122. To comment 4: In particular the scientific opinions on the safety of MON 89034, 1507 (renewal application) and MON 88017 were recently published. In addition, the EFSA GMO Panel received, at its request, updated bioinformatics-supported comparisons of the comparison of the sequences of the newly expressed Cry34Ab1, Cry35Ab1, and PAT proteins being present in maize 59122 with the sequences of known allergens and toxins (seen additional information received on 20 November 2009. No relevant similarities could be identified in the outcomes of these bioinformatics-supported studies.

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BELGIUM	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	1) Assessment of the allergenicity of the newly expressed proteins. It must be emphasized that Cry1A.105 displays high aminoacid sequence identity with Cry1Ac and that Cry1Ac has been proposed as an adjuvant for vaccines (Vasquez et al, 1999, Vasquez-Padron et al. 1999, Moreno-Fieros et al. 2003, Esquivel-Perez et al. 2005), which means that this protein is able to enhance the immune responses against antigens that are co-administered, which is not uncommon for a bacterial protein. Other proteins of the Cry family are also suspected of showing adjuvant properties (Calderon et al. 2007). Therefore, doubt may arise about Cry2Ab2, Cry1F, Cry3Bb1 and Cry34/35 Ab1. The consequence of the presence of such immuno-stimulant in a plant destined to human consumption is not known. Particularly the adjuvant effect via intestinal route is poorly documented. The single concentration of Cry1A.105 in maize grains is compatible with the possibility of an adjuvant effect in the context of normal maize grain consumption (but the concentration after processing of the maize or after cooking is not known). If all Cry proteins also have such adjuvant capacity, the adjuvant effect may be multiplied in MON89034x1507xMON88017x59122 maize. It is not known whether the presence of these Cry proteins in maize may elicit sensitization against the other maize proteins upon ingestion (and which type of sensitization?). This point needs to be clarified. Therefore, it is relevant to at least study in mice the immune responses against maize proteins when the animals are fed MON89034x1507xMON88017x59122 maize. 2) Assessment of the allergenicity of the whole GM plant or crop. The applicant did not assess the allergenicity of the whole GM plant. Care should be taken not to underestimate maize food allergy. Indeed, some maize allergens have been described in the literature (Pasini et al. 2002, Pastorello et al. 2003, Weichel et al. 2006, Fasoli et al. 2009) and, recently, patients showed maize-induced anaphylaxis in double-blind placebo-controlled food challenge, with reactions to as little as 100 mg of maize (Scibilia et al. 2008). This reinforces the need to evaluate	To comment 1: The EFSA GMO Panel is of the opinion that the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity caused by maize consumption or contact. Furthermore, maize is not a common allergenic food, and only a rare cause of occupational allergy may occur. The EFSA GMO Panel has considered the “weight of evidence” regarding potential allergenicity of MON 89034 x 1507 x MON 88017 x 59122 and its transgenic proteins, in line with its guidance and the internationally harmonized approach as described in *Codex alimentarius* guidelines. This weight of evidence also includes, besides the outcomes of the updated bioinformatics-supported comparisons and the issues previously considered in the evaluations of the single parental events (MON 89034, 1507, MON 88017, 59122), including the history of allergenicity, if any, of the sources of the transgenic proteins and the *in vitro* resistance of the transgenic proteins towards proteolytic enzymes. Also the potential unintended change in intrinsic allergenicity of the host maize has been considered in these opinions. To comment 2: Maize has not been officially classified as a major allergen (e.g. “the big eight”). Some of the considerations raised here are more general and do not specifically pertain to maize MON 89034 x 1507 x MON 88017 x 59122. Moreover, these considerations criticize the internationally harmonized approach recommended by *Codex alimentarius*, to which Belgium has also subscribed.

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			the allergenicity of the whole GM plant, as care must be taken that no increase in maize allergy incidence appears due to excessive allergen expression levels in modified maize. It is relevant to analyze whether the expression levels of known maize allergens is increased in the genetically modified maize grains or to analyze whether the overall allergenicity of the modified maize has increased, as compared to a natural counterpart. This is relevant as, theoretically, the introduction of all these new traits, through multiple cascade interactions, might have modified the expression level of some endogenous maize proteins. Patient IgE binding to modified maize grain extract or titration of known major allergens of maize should be carried out. The classical evaluation methods have been used and do not demonstrate the GMO to be a product which might be associated with allergy development. However, since the methods used are not completely predictive for allergy development long term follow up is warranted, e.g. the rapid digestibility in simulated digestive fluids is not a guarantee for safety. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein.	
BELGIUM	Belgian Biosafety Advisory Council	D, 07.09 Allergenicity	References for comments under D.7.9 1. Vásquez et al. Scand J Immunol 1999, 49:578-84 2. Vásquez-Padrón et al. Life Sci 1999, 64:1897-912 3. Moreno-Fiéros et al. Scand J Immunol 2003,57:45-55 4. Esquivel-Pérez et al. Viral Immunol 2005, 18:695-708 5. Calderón et al. Biologicals 2007 ; 35 :271-6. 6. Pasini et al. Allergy 2002; 57:98-106 7. Pastorello et al. J Allergy Clin Immunol 2003; 112:775-83 8. Weichel et al. Allergy 2006;61:128-35 9. Fasoli et al. J Proteomics 2009 ; 72 :501-10 10. Scibilia et al. Clin Exp Allergy 2008 ; 38 :1943-9 Bannon, G., Fu, T.J., Kimber, I., Hinton, D.M. 2003. Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111: 1122-1124.	References

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			Herman, R.A., Storer, N.P., Gao, Y. 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114: 1154-1157. Spök, A., Gaugitsch, H., Laffer, S., Pauli, G., Saito, H., Sampson, H., Sibanda, E., Thomas, W., van Hage, W., Valenta, R. 2005. Suggestions for the assessment of the allergenic potential of genetically modified organisms. Int. Arch. Allergy Immunol. 137:167-180.	
BELGIUM	Belgian Biosafety Advisory Council	D, 07.10 Nutritional assessment of GM food/feed	Comment 1 A broiler chicken feeding experiment with grain of the MON 89034x1507xMON 88017x59122 was performed. No negative effects were noted. The reported data were on pen level so that 10 replications, i.e. 5 pens per sex, were available. Based on the reported variability within treatments, the statistical power is not sufficient to find significant differences. Nevertheless based on the interpretation of the reported intervals of confidence, statistical significant differences were reported, but were not mentioned in the conclusions, because these were not considered as biological significant. However, it is worthwhile to mention that the SEM of some parameters was on average 3 times larger in the reported treatment groups than in the control, and that mortality rate was rather high. The calculation of the feed conversion ratio was not as exact as being possible. Comment 2 P.67 & 69 (Table 20) of the Technical Dossier: it is a pity that ADL is not included as fibre parameter. It is a pity that the nutritive value of MON 89034 × 1507 × MON 88017 × 59122 maize for different animals species (poultry, pigs and ruminants) is not reported, or the in vitro digestibility, as a parameterto indicate its nutritional equivalence.	To comment 1: The experimental setup of the chicken feeding study ([redacted]) was similar as that for many chicken feeding studies previously provided. Mortality was highest in the first days of the experiment, which apparently correlated with bacterial infection, dehydration and cervical dislocation (before group sizes were reduced from twelve to ten chicks per pen). Mortality in the second and longest part of the experiment was, on average, 1.9%. The EFSA GMO Panel describes the observed statistically significant differences in its opinion. In addition, it has requested from the applicants additional statistical data, *i.e.* a by-gender statistical analysis of the comparison between test and control (non-GM) dietary regimes. Both feed gain and adjusted feed gain have been provided in the results of the study. To comment 2: It is recognized that there is an ongoing discussion on which fiber parameters to use (e.g. at *Codex alimentarius* level). Given that the outcomes of the fiber analysis did not show any conspicuous effects of the genetic modification, no further analysis of different fiber parameters appeared to be warranted In the absence of changes in the nutritional profile of the product (e.g. based on compositional analysis), no further testing in target animals is needed

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				according to the case-by-case comparative assessment approach as formulated in EFSA and *Codex alimentarius* guidelines.
France	Ministère de l'Economie (Consommation)	General comments	Conclusion of the French Food Safety Agency The French Food Safety Agency (AFSSA) draws the following conclusions: The MON89034x1507xMON88017x59122 hybrid maize is obtained by conventional crossing of the genetically modified maize lines MON89034, 1507, MON88017 and 59122. Consequently, it expresses eight proteins encoded by the four inserts simultaneously. It can be concluded from the results of the chemical composition analysis that the genetically modified maize grain MON89034x1507xMON88017x59122 and its controls are substantially equivalent, except for the newly expressed proteins. Likewise, it can be concluded from the chicken feeding study that MON89034x1507xMON88017x59122 maize and its controls are nutritionally equivalent. The toxicological evaluation was conducted on maize containing each single transformation event. However, in the absence of convincing explanations as to the origin of the incidence of bladder calculi raised on examination of MON89034 maize or a sub-chronic toxicity study on the hybrid maize MON89034x1507xMON88017x59122, AFSSA cannot comment on the health safety of the maize grain MON89034x1507xMON88017x59122 and its derived products.	The issue of bladder calculi in the 90-days study with maize MON 89034 is discussed in the EFSA GMO Panel's opinion on this maize event, which was published in December 2008, as follows (taken from section 4.2.4): "*Microscopic findings in organs and tissues were almost equally distributed and no statistically significant differences between males and females of the high dose group and the controls were noted. A numerically higher incidence of kidney alterations in females of the high dose group was attributable to two rats (one died at day 14 of unknown cause, the other survived to the end of the study). The alterations in these two rats included minimal chronic progressive nephropathy, minimal/moderate transitional cell hyperplasia, minimal sub-acute inflammation and moderate hydronephrosis. The animal that died on day 14 additionally showed mild papillary necrosis and minimal tubular necrosis. Both rats had urinary bladder calculi and the study pathologist concluded that the lesions observed most likely were linked to these calculi. It seems unlikely that the urinary bladder calculi and associated kidney alterations could have been induced by the tested maize in 14 days. A low incidence of urinary bladder calculi is known to occur in this rat strain and may be considered a spontaneous finding in sub-chronic studies. According to historical control data supplied in the application, the incidence of urinary bladder calculi in high dose females in this study was also found in female control rats in previous studies conducted with CD rats in the same testing laboratory. The*

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				EFSA GMO Panel therefore considers the urinary bladder calculi as well as the associated kidney alterations as incidental findings which were not related to administration of maize MON 89034. The same applies to the nephroblastomas, a very rare tumour of the kidney, which were observed in two female animals of the control group." With regard to a 90-days study with the topical maize event MON 89034 x 1507 MON 88017 x 59122, no indications for unintended effects or substantial compositional changes have been observed that could warrant such a study (in accordance with the approach recommended by EFSA and *Codex alimentarius* guidance)

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France	Ministère de l'Economie (Consommation)	General comments	Conclusion de l'Agence Française de sécurité sanitaire des aliments L'Agence française de sécurité des aliments émet les conclusions suivantes : Les maïs hybrides MON89034x1507xMON88017x59122 sont obtenus par croisements conventionnels des lignées de maïs génétiquement modifiées MON89034, 1507, MON88017 et 59122. Par conséquent, ils expriment simultanément huit protéines codées par les quatre inserts. Les résultats de l'analyse de composition chimique permettent de conclure à l'équivalence en substance entre les grains de maïs génétiquement modifiés MON89034x1507xMON88017x59122 et leurs témoins, à l'exception des protéines nouvellement exprimées. De même, l'étude d'alimentarité réalisée chez le poulet permet de conclure à l'équivalence nutritionnelle des maïs MON89034x1507xMON88017x59122 et de leurs témoins. L'évaluation toxicologique a été conduite à partir des maïs comportant chaque événement de transformation simple. Cependant, en l'absence d'explications convaincantes sur l'origine de l'incidence des calculs vésicaux soulevée lors de l'examen du maïs MON89034 ou d'une étude de toxicité sub-chronique réalisées à partir des maïs hybrides MON89034x1507xMON88017x59122, l'AFSSA ne peut pas se prononcer sur la sécurité sanitaire des grains de maïs MON89034x1507xMON88017x59122 et de leurs produits dérivés.	(see response to English translation above)

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France	Ministère de l'Economie (Consommation)	A. General information	(A) General information The hybrid maize MON89034 x 1507 x MON88017 x 59122 was obtained by conventional crossing of genetically modified maize lines. The resultant MON89034x1507xMON88017x59122 maize is resistant to insects (European corn borer, cottonleaf worm, black cutworm, corn earworm, European corn borer and pink maize stalk borer, Western corn rootworm) and tolerant to herbicides (glyphosate and glufosinate-ammonium). It should be recalled that if this maize were to be imported, it would have to satisfy the regulations governing the use of herbicides.	The French comment referring to pesticide legislation is correct. However, as the scope of the present application excludes cultivation, environmental concerns related to the use of glufosinate-ammonium- and/or glyphosate-based herbicides on maize MON89034 x 1507 x MON88017 x 59122 apply only to imported and processed maize products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.
France	Ministère de l'Economie (Consommation)	A. General information	(A) Information générale Les maïs hybrides MON 89034 x 1507 x MON 88017 x 59122 ont été obtenus par croisements conventionnels des lignées de maïs génétiquement modifiées. Les maïs MON89034x1507xMON88017x59122 ainsi obtenus sont résistants à des insectes (pyrale, ver de cotonnier, noctuelle ipsilon, chenille des épis, pyrale et sésamie, chrysomèle des racines) et tolérant à des herbicides (glyphosate et glufosinate d'ammonium). Il convient de rappeler que ce maïs s'il venait à être importé devrait satisfaire à la réglementation relative à l'utilisation des herbicides	(see response to English translation above)
France	Ministère de l'Economie (Consommation)	D, 02 Information on the sequences actually inserted or deleted	(D) Information relating to the genetically modified plant (2) Information relating to the inserted or deleted sequences In its opinion of 20 November 2007 concerning the health safety of 89034 maize, AFSSA requested an extension of the sequencing of about 1000 bp on either side of the insertion so that the insertion site would be better characterised. In response to a similar request by EFSA, a supplementary study was supplied by the applicant in 2007 (1). This analysis of 2050 bp at 5' and 900 bp at 3' does not indicate that the insertion is produced in an endogenous maize gene. (1) Application EFSA-GMO-NL-2007-37 for authorisation of the	The EFSA GMO Panel agrees with this comment. Updated bioinformatic analyses of the 5' and 3' flanking sequences confirmed similarities to maize sequences and that the insert in MON 89034 is not located within any known endogenous maize gene.

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			genetically modified MON89034 maize submitted under Regulation (EC) No 1829/2003; replies to EFSA questions (Ref. SR/SM/shv (2008) out-2633518 13 February 2008 (21 January 2008).	
France	Ministère de l'Economie (Consommation)	D, 02 Information on the sequences actually inserted or deleted	(D) Informations relatives à la plante génétiquement modifiée (2) Information relatives aux séquences insérées ou délétées Dans son avis du 20 novembre 2007 relatif à la sécurité sanitaire des maïs 89034, l'AFSSA avait demandé une extension du séquençage d'environ 1000 pb de chaque côté de l'insertion afin que le lieu d'insertion soit mieux caractérisé. En réponse à une demande similaire de la part de l'AESA, une étude complémentaire a été fournit par le pétitionnaire en 2007 (1). Cette analyse de 2050 pb en 5' et de 900pb en 3' n'indique pas que l'insertion se soit produite dans un gène endogène de maïs. (1) Application EFSA-GMO-NL-2007-37 for authorization of the genetically modified MON89034 maize submitted under regulation (EC) N° 1829/2003 Responses to EFSA questions (Ref SR/SM/shv (2008) out-2633518 13 February 2008 (21 January 2008)	(see response to English translation above)
France	Ministère de l'Economie (Consommation)	D, 07.08 Toxicology	(7) Information relating to toxic and allergic effects and other adverse effects to human and animal health (7.8.2) Evaluation of subchronic toxicity A 13-week subchronic toxicity study was conducted in the rat on parental maize lines carrying the different events -MON89034, - 1507, - MON88017, or - 59122. The main aspects of these studies are mentioned in the AFSSA opinions of 20 November 2007 (2007-SA-0300), 28 January 2004 (2004-SA-0001), 4 April 2007 (2007-SA-0037) and 2 December 2007 (2007-SA-0303). It was concluded from the analysis of the results that the ingestion of	The issue of bladder calculi in the 90-days study with maize MON 89034 is discussed in the EFSA GMO Panel's opinion on this maize event, which was published in December 2008, as follows (taken from section 4.2.4): "*Microscopic findings in organs and tissues were almost equally distributed and no statistically significant differences between males and females of the high dose group and the controls were noted. A numerically higher incidence of kidney alterations in females of the high dose group was attributable to two rats (one died at day 14 of unknown cause, the other survived to the end of the study). The alterations in these two rats included minimal chronic progressive nephropathy, minimal/moderate*

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			maize grain carrying the different transformation events -1507, -MON88017- or - 59122 is without toxic effect in the rat exposed for 90 days via the diet. Conversely, in respect of the study conducted by ingestion of MON89034 maize (opinion of 20 November 2007, 2007-SA-0300), AFSSA asked for additional explanations to be provided about the difference in occurrence of bladder calculi between the historical data (0.49%) and the incidence of 10% (on the basis of 20 animals) observed in the female animals of the group ingesting the high dose of MON89034. Although historical data from 70 studies conducted between 1999 and 2006 with rats of the CD strain were submitted (letter sent to EFSA on 22/02/08), they are insufficient to conclude that there is no link between oral administration of MON89034 maize and the occurrence of bladder calculi observed in female animals fed with the high dose of MON89034. In view of the questions that still persist about this study, it would be necessary either to elucidate the results obtained or to present a specific toxicological study of the hybrid carrying the four transformation events. In addition, it would have been interesting to calculate a safety margin by means of the NOAELs that could be deduced from the 90-day subchronic toxicity studies and not from those deduced from the acute toxicity studies. (2) Application EFSA-GMO-NL-2007-37 for authorisation of the genetically modified MON89034 maize submitted under Regulation (EC) No 1829/2003; clarification on 90-day study – letter and attachment sent on 12 February 2008 to EFSA.	*transitional cell hyperplasia, minimal sub-acute inflammation and moderate hydronephrosis. The animal that died on day 14 additionally showed mild papillary necrosis and minimal tubular necrosis. Both rats had urinary bladder calculi and the study pathologist concluded that the lesions observed most likely were linked to these calculi. It seems unlikely that the urinary bladder calculi and associated kidney alterations could have been induced by the tested maize in 14 days. A low incidence of urinary bladder calculi is known to occur in this rat strain and may be considered a spontaneous finding in sub-chronic studies. According to historical control data supplied in the application, the incidence of urinary bladder calculi in high dose females in this study was also found in female control rats in previous studies conducted with CD rats in the same testing laboratory. The EFSA GMO Panel therefore considers the urinary bladder calculi as well as the associated kidney alterations as incidental findings which were not related to administration of maize MON 89034. The same applies to the nephroblastomas, a very rare tumour of the kidney, which were observed in two female animals of the control group.”*
France	Ministère de l'Economie (Consommati	D, 07.08 Toxicology	(7) Informations relatives aux effets toxiques, allergiques, et autres effets délétères pour la santé humaine et animale	(see response to English translation above)

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	on)		(7.8.2) Evaluation de la toxicité sub-chronique Une étude de toxicité sub-chronique a été réalisée durant 13 semaines chez le rat à partir des lignées parentales de maïs portant les différents événements -MON89034, - 1507, - MON88017, ou - 59122. Les éléments principaux de ces études sont indiqués dans les avis de l'AFSSA du 20 novembre 2007 (2007-SA-0300), du 28 janvier 2004 (2004-SA-0001), du 4 avril 2007 (2007-SA-0037), du 02 décembre 2007 (2007-SA-0303). L'analyse des résultats avait permis de conclure que l'ingestion de grain de maïs portant les différents événements de transformation -1507, -MON88017- ou - 59122 est sans effet toxique chez le rat exposé pendant 90 jours via l'alimentation. En revanche, concernant l'étude réalisée par ingestion des maïs MON89034 (avis du 20 novembre 2007, 2007-SA-0300), l'AFSSA avait demandé d'apporter des explications complémentaires sur la différence d'apparition des calculs dans la vessie entre les données historiques (0,49 %) et l'incidence de 10 % (base 20 animaux) observée chez les animaux femelles du groupe ayant ingéré la forte dose de MON 89034. Bien que des données historiques provenant de 70 études conduites entre 1999 et 2006 avec des rats de la souche CD, aient été transmises (courrier transmis à l'AESA le 22/02/08), elles ne sont pas suffisantes pour permettre de conclure à l'absence de lien entre l'administration orale de maïs MON89034 et la survenue des calculs de la vessie observés chez les animaux femelles nourries à la forte dose de MON89034. Compte tenu des interrogations qui subsistent sur cette étude, il serait nécessaire soit d'expliciter les résultats obtenus, soit de présenter une étude toxicologique spécifique de l'hybride portant les quatre événements de transformation. De plus, il aurait été intéressant de calculer une marge de sécurité	

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			grâce aux NOAEL pouvant être déduites des études de toxicité sub-chronique de 90 jours et non pas à partir de celles déduites des études de toxicité aigues. (2) Application EFSA-GMO-NL-2007-37 for authorization of the genetically modified MON89034 maize submitted under regulation (EC) N° 1829/2003 Clarification on 90 days study letter and attachment sent the 12th February 2008 to EFSA.	
Germany	Federal Agency for Nature Conservation (BfN)	General comments	The Federal Agency for Nature Conservation considers that further information is required before the risk assessment of EFSA/GMO/CZ/2008/62 can be finalised (see specific comments). Generally more studies based on plant derived materials of MON89034x1507xMON88017x 59122 maize are requested. MON89034x1507xMON88017x59122 maize combines several classes of Bt proteins active against both Lepidoptera and Coleoptera. For the environmental risk assessment interactions between these proteins should be addressed in more detail. MON89034x1507xMON88017x59122 maize also differs from the parental lines with regard to the absolute amount of toxin produced which is far greater than in the parental lines. We advise to reflect this stronger when assessing both health and environmental effects. In particular the information to assess expression, composition and phenotypic characteristics (both agricultural and ecological) of MON89034x1507xMON88017x59122 maize need to be expanded. The characterization of the GMO should be based on experiments in more than one planting season and the number of field sites should allow to test for possible gene-environment interaction including biotic and abiotic (climatic) factors in a statistically sound design. A major deficit of notification EFSA/GMO/CZ/2008/62 is the missing environmental exposure analysis and insufficient submission of studies to assess effects on non-target organisms. 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).	At the request of the EFSA GMO Panel. The applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.3, that "*The EFSA GMO Panel considered all the data available for maize MON 89034 x 1507 x MON 88017 x 59122 and the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) and is of the opinion that interactions between the maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x MON 88017 x 59122 are unlikely. Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary.*" The EFSA GMO Panel's guidance on the assessment of stacked events, section 3.2.2, notes that "*For the stacked events at least one year of field trial data is required, with trials performed together with appropriate controls in geographical localities representative of the climatic conditions under which such crops will be cultivated.*" The scope of the application includes food and feed

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			We want to point out that glufosinate, a complementary herbicide for use on MON89034x1507xMON88017x59122 maize will be phased out in Europe on September 30th 2017 because of its reproductive toxicity (see Annex I of Directive 91/414/EWG).	uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of these the individual events as present in its segregating progeny~~,~~ and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122, and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny) during transportation and processing. 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 89034 x 1507 x MON 88017 x 59122 during transportation and processing, except in the presence of glufosinate-ammonium- and/or glyphosate-based herbicides and/or under infestation by target pests. Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize MON 89034 x 1507 x MON 88017 x 59122 grains or through other routes indicate that the risk to non-target organisms is extremely low. It is highly unlikely that the recombinant DNA will transfer and establish in the genome of bacteria in the environment or human and animal digestive tracts. The intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 specifically exclude cultivation, and the environmental exposure to maize

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				MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events, as present in its segregating progeny, is limited to the accidental release of grains into the environment during transportation and processing. The EFSA GMO Panel considers that it would need successful establishment and spread of high numbers of maize MON 89034 x 1507 x MON 88017 x 59122 plants or their segregating progeny to enable any significant interaction with non-target organisms, which is very unlikely. In addition, the EFSA GMO Panel evaluated whether the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed grain produced by maize MON 89034 x 1507 x MON 88017 x 59122. Due to the specific insecticidal selectivity of the Cry proteins, non-target organisms most likely to be affected by the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins belong to the same or closely related taxonomic groups as those of the target organisms. Data supplied by the applicants suggest that only low amounts of the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins enter the environment due to low expression in grain. Moreover, these Cry proteins are degraded by enzymatic activity in gastrointestinal tracts of animals fed GM maize or derived feed products (see section 5.1.1), meaning that only low amounts of these proteins would remain intact to pass out in faeces. This has been demonstrated for Cry1Ab

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				(Einspanier et al., 2004; Guertler et al., 2008; Lutz et al., 2006; Lutz et al., 2005; Paul et al., 2009; Wiedemann et al., 2006). It is expected that there would subsequently be further degradation of Cry proteins in the manure and faeces due to intrinsic microbial proteolytic activity. Therefore, exposure of soil and aquatic environments to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1 Cry34Ab1 and Cry35Ab1 proteins from disposal of animal wastes or accidental spillage of maize grains is likely to be very low and localised. While Cry proteins may bind to a certain degree to clay minerals or humic substances in soil, thereby reducing their availability to microorganisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). Compared to the Cry1Ab protein, the Cry3Bb1 protein of GM maize was found to be degraded more rapidly in soil under similar conditions (Baumgarte and Tebbe, 2005; Miethling-Graff et al., 2010) Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny), it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins is likely to be very low and of no ecological relevance.

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Germany	Federal Agency for Nature Conservation (BfN)	D, 03 Information on the expression of the insert	Expression analysis must be regarded as an important part of the GMO risk assessment because it allows reflecting on the stability of the genetic modification, and indicates possible interactions between the GMO and environmental factors such as climate, soil or agricultural practice (e.g. fertilisation). Expression data should provide reliable estimates on the quantity of expression in different plant tissues with regard to biotic and abiotic factors. The data presented in the dossier do not meet the above objectives. Expression data were submitted from five North American sites for only one growing season (2006) (MSL-0021078; MSL- 0021070; 061026.05). No criteria were given for selecting the presented field sites which should be representative for a diversity of climatic and agronomic conditions. To complete the assessment of expression the notifier is asked to: • Provide information and selection criteria which allow to establish, that the chosen field sites are representative and cover a range of environmental and agronomic variables • Describe the chosen experimental sites in full detail indicating not only the region but the location of the field site. • Test differences between the stacked event and each of the parental lines in a statistically reliable design. • Test the influence of environmental factors such as climate or soil on expression in a statistically reliable design. • Test the influence of different genetic backgrounds on the expression pattern in a statistically reliable design. We strongly recommend comparing and analysing expression data with other data already available. We also recommend increasing sample size to allow analysing data with a higher statistical power. We also recommend testing the influence of the application of glyphosate and glufosinate on the expression. The expression data presented indicate that Cry1A.105 is expressed	The scope of the application covers food and feed uses, import and processing, therefore protein data related to the grain are considered most relevant and information on other tissues was provided. Expression data were supplied from trials conducted in 2006 at five location in the major USA maize growing regions that represent different environmental conditions. Expression levels were comparable to those of the single events. The EFSA GMO Panel is of the opinion that these data are sufficient from a safety point of view. The plants were treated with glufosinate-ammonium and glyphosate-based herbicides and this is considered sufficient as only grains from treated plants will be imported. The mean Cry1A.105 levels are indeed higher in grain of MON 89034 x 1507 x MON 88017 x 59122 compared to MON 89034. However there is an overlap in the range of Cry1A.105 levels measured in the stacked event and the single event MON 89034 and levels are low in grain and comparable to previously obtained results. It should be noted that differences in the levels of newly expressed proteins between stacked lines and the single events are not uncommon and do not necessarily pose a safety concern.

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			higher (twofold) in some tissues (pollen and grain) of the stacked GMO compared to the parental line 89034. Data also show that expression of PAT is markedly higher in all tissues of the stacked GMO than in the parental lines 59122 and 1507. While the increased expression of PAT can be explained by the additive action of the multiple gene copies present in the stacked GMO, the different expression pattern of Cry1A.105 in pollen and grain should be checked and further analysed.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.01 Comparative assessment	Data from the compositional analysis were presented from only one season (USA, 2006; based on production plan 06-01-52-04). Due to contamination of test material with other GMO only four of the five sites could be used for the compositional analyses. Compositional data for GMO without HR-treatment were not presented. The very limited number of sites (and climates) and years do not allow to test for possible effects of environmental variables. Since the compositional analysis presents a key element for the assessment of food/feed further data, including additional sites and years, should be presented.	The EFSA GMO Panel's guidance on the assessment of stacked events, section 3.2.2, notes that "*For the stacked events at least one year of field trial data is required, with trials performed together with appropriate controls in geographical localities representative of the climatic conditions under which such crops will be cultivated*." Given that the application is for import of maize, the locations chosen for the field trial appear to be well representative, in different parts of the major maize-growing areas in the USA. The EFSA GMO Panel considers that 4 sites chosen for this study are sufficient for the assessment of chemical composition for maize MON 89031 x 1507 x MON 88017 x 59122. The EFSA GMO Panel notes, in section 4.1.2. of the opinion, that "*Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides)*." Samples were taken from each replicate from maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart, and commercial maize varieties, then analysed for

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				composition. Grain samples were additionally checked for the presence of transgenic DNA by PCR. "*Due to the presence of recombinant DNA in grain of the conventional maize counterpart and one of the three commercial maize varieties at one location (which probably resulted from strong winds at the time of pollen shed), maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional maize counterpart, as well as the specified sample of the commercial maize variety from this location were not included in the final analysis. In consequence, the number of samples of forage and grain of either maize MON 89034 x 1507 x MON 88017 x 59122 or its conventional maize counterpart amounted to twelve (three per location, four locations in total), whilst fourteen commercial maize varieties from five locations were included.*" In section 4.1.3, it reads "*The EFSA GMO Panel considered the observed compositional differences between grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial maize varieties, and concludes that forage and grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 do not differ compositionally from its conventional counterpart and are equivalent to commercial maize varieties, except for the newly introduced traits.*".
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.04 Agronomic traits	Agronomic and phenotypic characterization The submitted study of Rosenbaum (2008; MSL-0021061) does not allow to conclude on possible ecological interactions of MON89034x1507xMON88017x59122 maize. For an assessment of ecological interactions with non-target organisms or in terms of pest and	Taking into account the scope of the application, both the rare occurrence of feral maize plants and low levels of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 protein exposure in maize MON 89034 x 1507 x MON 88017 x 59122 grains or through other routes indicate that the risk to target

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			disease incidence additional information and further field tests would be required. In this respect information on pest pressure, disease pressure or abiotic stressors have not been provided. Therefore, the baseline to compare stressor symptoms is missing. In fact data from Table 6 of MSL-0021061 indicate that pest pressure of the target organisms was week or absent. Moreover the application of different kinds of pesticides (see production plan) is counterproductive when assessing parameters listed in Tables 6 to 8. To conclude on the risk assessment the notifier is requested to i) to give the criteria on which the representativeness of locations has been established, ii) present further field data (several years, including treatments with and without HR) and to iii) statistically analyse the data giving the achieved statistical power. To allow conclusions on ecological characteristics of the GMO we strongly recommend including data from additional field seasons. We also recommend including data on the occurrence of volunteers during cultivation of MON89034x1507xMON88017x59122 maize to facilitate a better assessment of effects of loss and spillage.	and non-target organisms is extremely low.
Germany	Federal Agency for Nature Conservation (BfN)	D, 07.08 Toxicology	D.7.8.4. Testing of the whole GM food/feed Testing of the whole GM food/feed is crucial to obtain the necessary information about any adverse unintended effects of the stacked event MON89034x1507xMON88017x59122 maize on human or animal health. In this regard whole plant studies with the stacked GMO are especially important to test for unintended synergistic effects between the different Bt proteins and to account for the high absolute amount of Bt protein in food/feed derived from MON89034x1507xMON88017x59122 maize. However, the applicant's assessment of potential toxic effects of MON89034x1507x MON88017x59122 maize is mainly reduced to the risk assessment of the single events. Only one 42 day broiler chicken study has been carried out with the stacked event ([redacted]; MSL-0021066). However, this study was not designed to show possible toxicological effects but to show the effect of the genetic modification on broiler performance. The measured parameters are mainly of agricultural and economic relevance. In the broiler feeding study no	At the request of the EFSA GMO Panel. The applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.3, that "The EFSA GMO Panel considered all the data available for maize MON 89034 x 1507 x MON 88017 x 59122 and the newly expressed proteins (Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34/35Ab1, CP4 EPSPS, and PAT) and is of the opinion that interactions between the single maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x MON 88017 x 59122 are unlikely. Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary." It should be noted that the broiler feeding study is a

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			pathological or histopathological examinations are performed. Parameters of haematology and clinical biochemistry are not investigated. Hence this broiler feeding study cannot be regarded as a sufficient basis for toxicological risk assessment. As a consequence the safety of MON89034x1507xMON88017x59122 maize for human or animal health cannot be deduced from this study. To complete the risk assessment we recommend at least a 90-day oral toxicity study with rodents. In addition, we advise to carry out supplemental studies with ruminants and swine which differ with respect to their digestive systems and which will be substantially exposed to feed derived from MON89034x1507xMON88017x59122 maize.	nutritional study and not a toxicity study. In addition, no indications were identified that would warrant the performance of an animal toxicity study with the whole product. According to the approach outlined by the EFSA Guidance Document and the *Codex alimentarius* guidelines (to which also Germany has subscribed), animal safety tests and other tests with GM plant-derived foods are not required *per se* but on a case-by-case basis, based on indications, for example, of certain unintended effects or substantially modified composition. Given the EFSA GMO Panel's conclusion that interactions that might impact on safety are unlikely, there is no need to carry out such studies
Germany	Federal Agency for Nature Conservation (BfN)	D, 10.05 Interactions of the GM plant with non-target organisms	Exposure analysis An environmental exposure analysis and therefore a key for the assessment of effects on NTO is missing. Although the notifier acknowledges (but not quantifies) exposure via loss and spillage the possibility that the GMO or derived material enter the environment has not been considered. The main purpose of application EFSA-GMO-CZ-62 is the use for food and feed uses and a main exposure route therefore will result from feeding the GMO to livestock and wild animals. The notifier is requested to submit a detailed exposure analysis including the exposure of the environment via the food-feed chain, including the exposure of soil and water to Bt proteins. Data on the quantity and the degradation of the mixture of Bt proteins in all relevant media such as organic waste, waste water, and manure are required. Following this, the potential accumulation of the Cry proteins in the environment should be assessed.	The scope of the application is for food and feed uses, import and processing of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of these the individual events as present in its segregating progeny, and does not include cultivation. Considering the proposed uses of maize MON 89034 x 1507 x MON 88017 x 59122, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed grain (F_2 generation) produced by maize MON 89034 x 1507 x MON 88017 x 59122 and with the accidental release into the environment of viable grains from maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny see section 3.1) during transportation and processing.

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Germany	Federal Agency for Nature Conservation (BfN)	D, 10.05 Interactions of the GM plant with non-target organisms	NTO In addition to the exposure analysis data on the eco-toxicity of MON89034x1507x MON88017x59122 maize are required to assess possible effects on non-target organisms and subsequent effects on biogeochemical processes. We want to stress here that experiments with MON89034x1507xMON88017x59122 maize should be carried out. Data from the parental lines can be informative but not sufficient for the risk assessment (see Andow & Hilbeck 2004; Hilbeck et al 2008). Experiments should account for the high total amount of Bt protein in MON89034x1507xMON88017x59122 maize and for possible interactions of the mixture of Cry1.105, Cry2Ab2, Cry1F, Cry3Bb1 and Cry34Ab1/Cry35Ab1. The two studies submitted on possible interactions of Cry Proteins (MacRae 2008 and Levine et al. 2008) focus on the target organisms. Known differences between the sensitivity to Bt-Toxin within the taxonomic order related to the target organisms as well as methological details of the studies do not allow to assess interactions of the present Bt proteins in general. Because the scope of EFSA-GMO-CZ-2008-62 does not include cultivation, soil and water organisms are the most likely organism groups for which unexpected effects have to be assessed. Exposure routes, functional groups and test species should be selected in accordance with an ecological test strategy (Hilbeck et al. 2008). Having collected data on the ecotoxicity the risk assessment should be updated including possible effects on soil and water organisms. Special attention should be paid to unexpected effects on water organisms. Several recent publications point at the presence of Cry Proteins and/or genes in aquatic systems and raise concerns about the safety of plant expressed Cry-Proteins to aquatic organisms (Bøhn et al. 2008; Douville et al. 2005, 2008; Prihoda & Coats, 2008; Rosi-Marshall et al. 2007). Experiments on caddisfies (Rosi-Marshall et al. 2007), midges (Prihoda & Coats, 2008), water fleas (Bøhn et al. 2008) as well as ladybird beetles (Schmidt et al. 2008) or nematodes (Höss et al. 2008) point out, that Bt proteins may be less specific than assumed and that the mode of action may differ from target insects (see also Hilbeck & Schmidt 2006; Schmidt et al. 2009).	Considering the scope of the application (that excludes cultivation) and the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 (which include its segregating progeny), it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins is likely to be very low and of no ecological relevance.

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Germany	Federal Agency for Nature Conservation (BfN)	D, 10.05 Interactions of the GM plant with non-target organisms	Andow,D.A. & Hilbeck,A. (2004) Science-based risk assessment for non-target effects of transgenic crops. BioScience, 54, 637-649. Bøhn,T., Primicerio,R., Hessen,D.O. & Traavik,T. (2008) Reduced Fitness of Daphnia magna Fed a Bt-Transgenic Maize Variety. Arch Environ Contam Toxicol, currently online (DOI 10.1007/s00244-008-9150-5). Douville,M., Gagné,F., Masson,L., McKay,J. & Blaise,C. (2005) Tracking the source of Bacillus thuringiensis Cry1Ab endotoxin in the environment. Biochemical Systematics and Ecology, 33, 219-232. Douville,M., Gagné,F. & Blaise,C. (2008) Occurrence of the transgenic corn cry1Ab gene in freshwater mussels (Elliptio complanata) near corn fields: Evidence of exposure by bacterial ingestion. Ecotoxicology and Environmental Safety (online), 1-9. Hilbeck A., Jänsch, S., Meier M., Römbke J. (2008b) Analysis and validation of present ecotoxicological test methods and strategies for the risk assessment of genetically modified plants. Federal Agency for Nature Conservation, Bonn - Bad Godesberg: 287 pp. (BfNSkript 236) http://www.bfn.de/fileadmin/MDB/documents/service/skript236.pdf Hilbeck,A. & Schmidt,J.E.U. (2006) Another view on Bt proteins - how specific are they and what else might they do? Biopesticides International, 2, 1-50. Höss,S., Arndt,M., Baurngarte,S., Tebbe,C.C., Nguyen,H.T. & Jehle,J.A. (2008) Effects of transgenic corn and CrylAb protein on the nematode, Caenorhabditis elegans. Ecotoxicology and Environmental Safety, 70, 334-340. Prihoda,K.R. & Coats,J.R. (2008) Aquatic fate and effects of Bacillus thuringiensis Cry3Bb1 protein: toward risk assessment. Environmental Toxicology and Chemistry, 27, 793-798. Rosi-Marshall,E.J., Tank,L.J., Royer,T.V., Whiles,M.R., Evans-White,M., Chambers,C., Griffiths,N.A., Pokelsek,J. & Stephen,M.L. (2007): Toxins in transgenic crop byproducts may affect headwater stream ecosystems. Proceedings of the National Academy of Science USA, 104, 16204-16208. Schmidt,J.E.U., Braun,C.U., Whitehouse,L.P. & Hilbeck,A. (2009) Effects of Activated Bt Transgene Products (Cry1Ab, Cry3Bb) on Immature Stages of the Ladybird Adalia bipunctata in Laboratory	References

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			Ecotoxicity Testing. Arch Environ Contam Toxicol, 56, 221-228.	
Germany	Federal Agency for Nature Conservation (BfN)	D, 10.08 Effects on biogeochemical processes	Data on the degradation of Cry toxins during processing and the use of food/feed for MON89034x1507xMON88017x59122 are missing. With respect to studies on the degradation of microbially derived Cry1A.105, CryAb2, Cry1F, Cry3Bb1 and Cry34Ab1/Cry35AB1 the notifier is requested to refer to scientific studies and not to other EFSA dossiers (e.g. as done in page 143 of the dossier). As stated in the EFSA guidelines applications need to be stand-alone documents. To assess the degradation of Cry Proteins detailed description of the used methodology is necessary. The cited half-lifes of Cry Toxins by the notifier seem to be in conflict with results from peer reviewed literature (e.g. Crechio & Stotzky 1998, 2001; Hönemann et al. 2008, Rauschen et al. 2008; Tapp & Stotzky 1998; Zwahlen et al. 2003). Crecchio, C. & Stotzky, G. (1998) Insecticidal activity and biodegradation of the toxin from Bacillus thuringiensis subsp. kurstaki bound to humic acids from soil. - Soil Biol. Biochem. 30: 463-470. Crecchio, C. & Stotzky, G. (2001) Biodegradation and insecticidal activity of the toxin from Bacillus thuringiensis subsp. kurstaki bound on complexes of montmorillonite-humic-acids-Al hydroxy-polymers. - Soil Biol. Biochem. 33: 573-581. Hönemann,L., Zurbrügg,C. & Nentwig,W. (2008) Effects of Bt-corn decomposition on the composition of the soil meso- and macrofauna. Applied Soil Ecology, 40, 203-209. Rauschen,S., Nguyen Thu,H., Schuphan,I. & et al. (2008) Rapid degradation of the Cry3Bb1 protein from Diabrotica-resistant Bt-corn MON88017 during ensilation and fermentation in biogas production facilities. J Sci Food Agr, 88, 1709-1715. Tapp, H. & Stotzky, G. (1998) Persistence of the insecticidal toxin from Bacillus thuringiensis subsp. kurstaki in soil. Soil Biol. Biochem. 30: 471-476. Zwahlen,C., Hilbeck,A., Gugerli,P. & Nentwig,W. (2003): Degradation of the Cry1Ab protein within transgenic Bacillus thuringiensis corn tissue in the field. Molecular Ecology, 12, 765-775.	It is noted that *Codex alimentarius* recommends the performance of *in vitro* resistance test against proteolysis by pepsin, which has been performed for the newly expressed proteins in each single event (see the EFSA GMO Panel's opinions on each of these events)

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Germany	Federal Agency for Nature Conservation (BfN)	D, 12.01 General	As stated by the notifier, the scope of the application of MON 89034 × 1507 × MON 88017 × 59122 maize is for import, processing and all uses for food and feed. The notifier's proposal for an environmental monitoring plan does not fully meet the requirements defined in Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC. The provided monitoring plan remains very general and needs further specification. Therefore, a detailed and meaningful monitoring plan suitable to meet the objectives defined in Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC is requested. A clear concept of data collection, analysis and evaluation is missing. Adverse effects on the environment cannot be identified unless protection goals are determined. Monitoring parameters have to be selected with reference to these protection goals. The step of data recording, the step of subsequent data analysis and the last step of evaluating the results have to be separated. Only the evaluation step can answer the question whether adverse effects have occurred with respect to the predefined protection goals. The notifier is requested to revise the monitoring plan according to these requisites. Monitoring the environmental effects of MON89034x1507xMON88017x59122 maize should serve as an early warning system: The data which will be collected should be relevant to and suitable for a "rapid assessment and implementation of measures to reduce any consequences to the environment" (Council Decision 2002/811/EC). In order to assess, whether the monitoring plan is appropriate to fulfil this task, the following requirements have to be met: • A fully specified list of monitoring parameters has to be provided. The notifier is requested to present for each parameter a detailed statement of the parameter definition, the observation methods (collection and analysis of samples with references), the frequencies of observations (time and number of visits to collect data) and the monitoring locations including number and size. • The operating schedule giving full details of points in time is requested.	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.

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			• The methods of data analysis including the statistical methods have to be elaborated. It must be explained how, within the sampling concept, the necessary representativeness of the collected data in space and time is ascertained. • The baseline status of the receiving environment with respect to the monitoring parameters has to be determined and reference areas have to be characterised. • The notifier is requested to indicate how the monitoring plan is adapted to different local conditions where appropriate. • To ensure the compliance with fundamental quality criteria and the comparability of monitoring data from different regions and EU Member States, standard methodology should be followed where appropriate (e.g. CEN, OECD-Methods or VDI Guidelines). • In case of monitoring data being collected by external persons or institutions other than the notifier, binding agreements/contracts with third parties are requested which clearly determine which data will be provided and how these data will be made available. The monitoring should be run in regions, where MON89034x1507xMON88017x59122 maize willbe transported, stored, processed and used.	

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Germany	Federal Agency for Nature Conservation (BfN)	D, 12.02 Case-specific GM plant monitoring	According to the applicant incidental spillage of MON89034x1507xMON88017x59122 maize during transport, storage, packaging or processing can occur. Therefore, a case-specific monitoring is necessary and has to focus on pathways, how the MON89034x1507xMON88017x59122 maize can enter the environment. The case-specific monitoring plan has to comprise the exposure of the environment to MON89034x1507xMON88017x59122 maize kernels e.g. via spillage during transport, storage, packaging, processing and use. Furthermore the case specific monitoring has to focus on the exposure of organic waste material, sewage or by-products containing MON89034x1507xMON88017x59122 Cry proteins to the environment during or after the production process or animal consumption.	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.03 General Surveillance of the impact of the GM plant	According to Directive 2001/18/EC general surveillance is a compulsory part of the monitoring. The objective of general surveillance is to monitor potential cumulative long-term impacts on human health and the environment and to identify the occurrence of adverse effects of the GMO on human health and the environment which were not anticipated in the environmental risk assessment. The notifier states that the general surveillance will be mainly based on information gathered from the existing networks of COCERAL, UNISTOCK and FEDIOL. These are European organisations which represent national organisations. Data shall be collected by operators. Communication with operators will be partly delegated to EuropaBio. An important tool for information exchange will be the website hosted by EuropaBio. It is not clear, how the listed European organisations and EuropaBio will inform and instruct operators about their surveillance function. Therefore, the notifier is requested to state who is responsible to organise the information exchange from the European over the national to the local level and to name the national and local	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section

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			organisations and factories. Furthermore, the notifier is requested to state how it can be ascertained that operators agree to contribute to the general surveillance. Since the general surveillance conducted by operators has to address environmental impacts of MON 89034 × 1507 × MON 88017 × 59122 maize, the notifier is requested to explain how it will be ascertained that the operators in duty for general surveillance show the necessary skills to identify environmental impacts of MON 89034 × 1507 × MON 88017 × 59122 maize. Monitoring parameters and approaches for general surveillance are not specified at all. Furthermore, protection goals are not referred to. The description of the elements of the general surveillance plan remains too general.	6.1.3 of the scientific opinion.
Germany	Federal Agency for Nature Conservation (BfN)	D, 12.06 Reporting the results of monitoring	The monitoring results have to be reported on an annual basis. All raw data have to be provided upon request. The notifier should use the monitoring format provided by the Commission and agreed on by the Member States. According to Directive 2001/18/EC (Art. 20 number 4), the results of the monitoring carried out under part C of the Directive shall be made publicly available. Therefore, the notifier is requested to state, how the monitoring results will be published.	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.
Germany	Federal Office of Consumer Protection	General comments	The scope of application EFSA-GMO-CZ-2008-62 covers import and processing of maize MON 89043 x 1507 x MON 88017 x 59122 including all feed and food products containing, consisting of, or	

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	and Food Safety		produced from the genetically modified maize MON 89043 x 1507 x MON 88017 x 59122. 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 with the application EFSA-GMO-CZ-2008-62 support the conclusion that maize MON 89043 x 1507 x MON 88017 x 59122 is unlikely to have adverse effects on human and animal health or on the environment in the context of its intended use. However, clarification on some points of the dossier is necessary to conclude on the risk assessment. In line with the risk assessment of maize MON 89043 x 1507 x MON 88017 x 59122 the applicant refers to data given in the respective applications for authorization of the single events MON 89034 (see EFSA-GMO-NL-2007-37), 1507 (see EFSA-GMO-NL-2004-02), MON 88017 (see EFSA-GMO-CZ-2005-27) and 59122 (see EFSA-GMO-NL-2005-12), respectively. Therefore, we would like to refer to the German comments which we have already submitted in conjunction with the risk assessment of these applications. In this regard, we ask to consider the pending points also within the evaluation of application EFSA-GMO-CZ-2008-62. Moreover, in order to facilitate a detailed examination of all relevant material, we would like to propose that appropriate data of the original single event applications as well as additionally provided information should be clearly represented within the application documents of dossier EFSA-GMO-CZ-2008-62. Specification of the plan for general surveillance is requested as the objectives defined in Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC are not fully met.	The single events 1507, 59122, MON 88017 and MON 89034 have been the subjects of previous assessments and have received an EFSA opinion in favour of their authorisation (EFSA, 2004, 2005a, b, 2007, 2008, 2009a, b). The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.
Germany	Federal Office of Consumer Protection and Food Safety	A, 07 Where appropriate, the conditions for placing on the market the	The import documents should indicate that maize MON 89043 x 1507 x MON 88017 x 59122 has not been approved for cultivation by the EC. Appropriate measures have to be taken during transport, storage, and processing to avoid unintended release into the environment.	The EFSA GMO Panel takes into account that this application does not include cultivation of maize MON 89034 x 1507 x MON 88017 x 59122 within the EU so that the likelihood of cross-pollination between cultivated maize and the occasional feral maize plants resulting from grain spillage is considered extremely low. However, in countries

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		food(s) or		cultivating maize MON 89034 x 1507 x MON 88017 x 59122 and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of maize MON 89034 x 1507 x MON 88017 x 59122 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.
Germany	Federal Office of Consumer Protection and Food Safety	D, 07.02 Field trials	D.7.2. Production of material for comparative assessment: Production of material for comparative assessment was conducted at four replicated field sites in major maize-growing areas of the U.S.A. during the 2006 field season. According to production plan # 06-01-52-04 (Monsanto Company and Dow AgroSciences LLC, 2007) all test plots received applications of glyphosate as well as of glufosinate-ammonium. In accordance with the EFSA Guidance Document (EFSA, 2006) we would like to stress that in the case of herbicide tolerant GM plants both blocks of genetically modified plants exposed to the intended herbicide(s) and blocks not exposed to the herbicide(s) should be included with regard to the production of material for comparative assessment. This design would allow assessment of whether the expected agricultural condition might influence the expression of the studied parameters within the compositional analysis as well as the analysis of agronomic traits. Therefore, the applicant should be requested to demonstrate that forage and grain from maize MON 89043 x 1507 x MON 88017 x 59122 are compositionally equivalent to and as nutritious as forage and grain from conventional maize regardless of herbicide treatment. This applies analogously to the comparative assessment of the phenotypic, agronomic, and ecological characteristics of maize MON 89043 x 1507 x MON 88017 x 59122. 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.	The EFSA GMO Panel notes, in section 4.1.2. of the opinion, that "*Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides)*."Samples were taken from each replicate from maize MON 89034 x 1507 x MON 88017 x 59122, its conventional counterpart, and commercial maize varieties, then analysed for composition. Grain samples were additionally checked for the presence of transgenic DNA by PCR. Due to the presence of transgenic DNA in grain of the conventional maize counterpart and one of the three commercial maize varieties in one location, which probably related to pollen flow between adjacent fields under strong wind conditions, maize MON 89034 x 1507 x MON 88017 x 59122 and its conventional maize counterpart from this location were not included in the final analysis. This also pertained to one of the three commercial maize varieties grown in the same location. In

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			Monsanto Company and Dow AgroSciences LLC. (2007) Field production report: A U.S. field production of corn grain and tissues from NK603, MON 89034, TC1507, MON 88017, DAS-59122-7, conventional crosses MON 88017 x DAS-59122-7, MON 89034 x MON 88017 x DAS-59122-7, MON 89034 x TC1507 x NK603, MON 89034 x TC1507 x MON 88017 x DAS-59122-7, and a conventional control during 2006 (production plan #: 06-01-52-04), Monsanto Technical Report, MSL-0021078.	consequence, the number of samples of forage and grain of either maize MON 89034 x 1507 x MON 88017 x 59122 or its conventional maize counterpart amounted to twelve (three per location, four locations in total), whilst 14 commercial maize varieties from five locations were included"
Germany	Federal Office of Consumer Protection and Food Safety	D, 07.07 Anticipated intake/extent of use	Maize MON 89034 × 1507 × MON 88017 × 59122 is to be used as any other maize in the E.U. including the production of foodstuff ingredients. 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. Moreover, information on known or anticipated human/animal exposure to other sources of analogous GM food/feed and from other routes of exposure to the new gene products is missing and should be provided. In this context, an exposure assessment regarding the new gene products taking into account all sources of exposure is lacking and might be requested from the applicant.	Taking into account that no risk has been identified and that a Pan-European database on consumption data is not yet available, and that the estimated exposure is very low, a more detailed exposure assessment appears not to be warranted. The data in the dossier containing an estimate of potential exposure to the transgenic proteins can be summarized as follows: Based on the expression levels of the newly expressed proteins measured during the field trials in the USA in 2006, and on human and animal consumption data for maize and derived products, the applicants estimated the potential intake of the newly expressed proteins by humans and animals consuming maize. Whilst the estimates were conservative, assuming a 100%-substitution scenario and no losses of newly expressed proteins during processing, the outcomes show that these levels were several orders of magnitude below the levels having no adverse effects in the acute oral toxicity studies previously performed with these proteins.
Germany	Federal Office of Consumer Protection and Food Safety	D, 07.08 Toxicology	D.7.8.1. Safety assessment of newly expressed proteins The applicant evaluated the potential for interactions between the coleopteran-active proteins Cry3Bb1, Cry34Ab1 and Cry35Ab1 on the one hand, and between the lepidopteran-active proteins Cry1A.105, Cry2Ab2 and Cry1F on the other hand by insect bioassays. Moreover, the applicant evaluated whether combined Cry1A.105, Cry2Ab2 and	At the request of the EFSA GMO Panel. The applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.3, that "*The EFSA GMO Panel considered all the data available for maize*

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			Cry1F activity is altered by the presence of the Cry3Bb1 and the Cry34/35Ab1 binary proteins. However, a statement whether combined Cry3Bb1, Cry34Ab1 and Cry35Ab1 activity is altered by the presence of the Cry1A.105, Cry2Ab2 and Cry1F proteins is missing. Thus, for the sake of completeness, the applicant should be requested to complete the evaluation in this respect.	*MON 89034 x 1507 x MON 88017 x 59122 and the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) and is of the opinion that interactions between the maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x MON 88017 x 59122 are unlikely. Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary*." It should be noted that the safety of the Cry proteins for humans and animals is different from that for target insects. The EFSA GMO Panel writes, in section 5.1.4.3 that "*Maize MON 89034, 1507, MON 88017 and 59122 have previously been found as safe as their conventional counterparts for human and animal consumption (EFSA, 2004, 2005a, 2005b, 2007, 2008, 2009a, 2009b). As described in section 5.1.1, the EFSA GMO Panel's assessment of the single maize events MON 89034, 1507, MON 88017, and 59122 also considered the outcomes of 90-days rat feeding studies with each of these single events, which did not show adverse treatment-related effects (EFSA, 2004, 2005a, 2005b, 2007, 2008, 2009b). In the present assessment, no change in the structural integrity of the inserts in maize MON 89034 x 1507 x MON 88017 x 59122 was found when compared to the respective single events in the analysis of molecular characteristics, and protein levels of grain produced from maize MON 89034 x 1507 x MON 88017 x 59122 were shown to be comparable to those in the respective single maize events (see section 3.2). Moreover, the compositional, agronomic and phenotypic characteristics of maize*

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				MON 89034 x 1507 x MON 88017 x 59122 were not different from those of its conventional counterpart (see section 4.2). In addition, at the EFSA GMO Panel's request, the applicant provided an assessment of the potential interactions between the events combined within maize MON 89034 x 1507 x MON 88017 x 59122 that could impact on human and animal health2. The EFSA GMO Panel considered all the data available for maize MON 89034 x 1507 x MON 88017 x 59122 and the newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) and is of the opinion that interactions between the maize events that might impact on the food and feed safety of maize MON 89034 x 1507 x MON 88017 x 59122 are unlikely. Therefore, the EFSA GMO Panel does not consider additional animal safety studies with the whole GM food/feed necessary." In section 5.1.4.1, it is noted that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.*"

[2] Additional info June 2009

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Germany	Federal Office of Consumer Protection and Food Safety	D, 12 Environmental Monitoring Plan	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. According to the risk assessment no adverse effects on the environment or human health were identified or were expected. Therefore, there is no necessity for a case-specific monitoring. 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 needs 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 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	The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.

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			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 General Surveillance 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.	
Italy	Ministero dell'Ambiente e della Tutela del Territorio	General comments	Notifier should complete the documentation supplied regarding: - The information on the genetic stability and the toxicity of each single event; - The risk assessment of potential interactions among the 8 newly proteins expressed in the event, taking into account that the aspects related to the possible effects on human and animal health has not been addressed; - The proposal of the surveillance plan in which the approaches and the introduced methodologies of analysis proposed do not bring to light the critical points of the monitoring. Especially, should be considered (a) the frequency of the possibility of GM living material dispersion and the frequency of the presence of volunteers, when the material arrive at the port and (b) phases of the working to obtain the final product.	The molecular data supplied by the applicants do not suggest a structural modification due to the conventional crossing of the single events in the stacked lines. The stability of the single events was demonstrated over several generations, stability of the stacked event over one generation. This is considered to be sufficient from a safety point of view. At the request of the EFSA GMO Panel, the applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion (section 5.1.4.1) that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety*

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				concern." The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicants is in line with the intended uses of maize MON 89034 x 1507 x MON 88017 x 59122 and all sub-combinations of the individual events in the segregated progeny, as the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and scientific opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholder, including national competent authorities. The information supplied by the applicants is in line with this guidance. See section 5.2 of the PMEM opinion (EFSA, 2006b) and section 6.1.3 of the scientific opinion.
Norway	Directorate for nature management	General comments	The Norwegian CA request the Notifier to list all third countries where applications for MON 89034 x 1507 x MON 88017 x 59122 and the parental lines 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 89034 x 1507 x MON 88017 x 59122.	This is outside the scope of the EFSA GMO Panel.

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Norway	Directorate for nature management	General comments	The Norwegian CA requests the Notifier to provide further information that will allow the Norwegian authorities to evaluate the possible contributions of maize MON 89034 x 1507 x MON 88017 x 59122 to a sustainable development, benefits 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/2005/regulations-relating-to-impact-assessmen.html?id=440455 Of primary interest are changes in pesticide use the cultivation of MON 89034 x 1507 x MON 88017 x 59122 are foreseen to lead to. Given the presence of the epsps and pat genes, it seems likely to assume that cultivation of MON 89034 x 1507 x MON 88017 x 59122 will lead to increased use of glyphosate and glufosinate herbicides in maize production. The presence of multiple cry genes in MON 89034 x 1507 x MON 88017 x 59122 could potentially result in changes in use of insecticides. The Notifier should elaborate further on environmental effects and effects on human health (positive or negative) of the changes in agricultural practices due to: a) increased use of glyphosate and glufosinate; and b) the shift from herbicides used presently to glyphosate and glufosinate. The Notifier should also elaborate on the effects on foreseen changes in insecticide use resulting from cultivation of MON 89034 x 1507 x MON 88017 x 59122.	This is outside the scope of the EFSA GMO Panel. As the scope of the present application excludes cultivation, environmental concerns related to the use of glufosinate-ammonium- and/or glyphosate-based herbicides on maize MON89034 x 1507 x MON88017 x 59122 apply only to imported and processed maize products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.
Norway	The Norwegian Scientific Committee for Food Safety	D, 05 Genetic stability of the insert and phenotypic stability of the GM	Stacked events: The applicant is asked to test the maize for genetic stability of the inserts for more than one generation, e.g. three growing season and multiple locations representing different environmental conditions.	The molecular data supplied by the applicants do not suggest a structural modification due to the conventional crossing of the single events in the stacked lines. The stability of the single events was determined over several generations, stability of the stacked event over one generation. This is considered to be sufficient from a safety point of view.

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		plant		Furthermore, maize hybrid lines are only grown for one generation therefore the analysis should be carried out on this generation. The agronomic characteristics of MON 88017 x MON 89034 x 11507 x 59122 together with the compositional analysis did not raise any concerns over unintended effects. Weight of evidence, therefore, indicates no safety concerns.
Norway	The Norwegian Scientific Committee for Food Safety	D, 07.01 Comparative assessment	The expression of the cry1A.105 gene in MON 89034 x 1507 x MON 88017 x 59122 is about 100 % higher in pollen and about 50 % higher in corn compared to MON 89034. The applicant is asked to explain these differences.	The scope of the application covers food and feed uses, import and processing, therefore only protein data related to the grain are considered relevant. The mean Cry1A.105 levels are indeed higher in MON 89034 x 1507 x MON 88017 x 59122 compared to MON 89034. However there is an overlap in the range of Cry1A.105 levels measured in stacked event and MON 89034. It should be noted that differences in the levels of newly expressed proteins between stacked lines and the single events are not uncommon and do not necessarily pose a safety concern.
Norway	The Norwegian Scientific Committee for Food Safety	D, 07.02 Field trials	According to the EFSA Guidance Document for the risk assessment of GM plants, it is advisable that experiments with herbicide tolerant crops "include both blocks of genetically modified plants exposed to the intended herbicide and blocks not exposed to the herbicide". In the study report on the compositional analyses it is not indicated whether MON 89034 x 1507 x MON 88017 x 59122 maize plots were treated with glyphosate/glufosinate. The applicant is asked to clarify whether treatments with glyphosate/glufosinate were performed, and to include compositional data from MON 89034 x 1507 x MON 88017 x 59122 maize treated and not treated with glyphosate/glufosinate.	In section 4.1.2 describing the field trial design for the comparative analysis, the EFSA GMO Panel's opinion addresses the issue of herbicide treatment with the target herbicides as follows: "*Given the fact that previous assessments of the herbicide-tolerant single events MON 88017, 1507 and 59122 considered both plants treated with the target and conventional herbicides and plants treated with only conventional herbicides, the EFSA GMO Panel does not consider it necessary to ask for compositional data on maize MON 89034 x 1507 x MON 88017 x 59122 that was treated with conventional herbicides (i.e. not with the target herbicides)*." The herbicide treatment is described in more detail in the appendix production plan 06-01-52-04. In

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				addition, in response to a query by the EFSA GMO Panel, the applicants have explained that the doses of glufosinate-ammonium and glyphosate-based herbicides are representative of those used in commercial practice.
Norway	The Norwegian Scientific Committee for Food Safety	D, 07.09 Allergenicity	7.9.2 Assessment of allergenicity of the whole GM plant or crop. Scientific studies, also very recent ones, have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant, which is an enhancer of immune responses. The GMO Panel of the Norwegian Scientific Committee for Food Safety find it difficult, based on the available data, to assess whether kernels from maize MON 89034 x 1507 x MON 88017 x 59122 may cause more allergenic reactions than food and feed from unmodified kernels. As the different Cry proteins are closely related, and in view of the experimental studies in mice, the GMO Panel finds that the likelihood of an increase in allergenic activity due to Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins in food and feed from maize MON 89034 x 1507 x MON 88017 x 59122 cannot be excluded. Thus, the Panel's view is that as the adjuvant effect of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 with reasonable certainty cannot be excluded, the applicant in relation to a possible adjuvant effect of Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 must comment upon the mouse studies showing humoral antibody response of Cry1A proteins. Further, although Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1 and Cry35Ab1 proteins is rapidly degraded in gastric fluid after oral uptake, there is also the possibility that the protein can enter the respiratory tract after exposure to e.g. mill dust. Finally, rapid degradation is no absolute guarantee against allergenicity or adjuvanticity. References: Moreno-Fierros L, Ruiz-Medina EJ, Esquivel R, López-Revilla R, Piña-Cruz S., 2003. Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand J Immunol., 57: 45-55 Prasad S.S.S.V. & Shethna, Y.I., 1975. Enhancement of immune response by the proteinaceous crystal of Bacillus thuringiensis var	The EFSA GMO Panel is of the opinion that the adjuvant effect of Cry proteins, observed after high dosage intragastric or intranasal administration will not raise any concerns regarding allergenicity caused by maize consumption or contact. Furthermore, maize is not a common allergenic food, and only a rare cause of occupational allergy may occur. The EFSA GMO Panel has considered the “weight of evidence” regarding potential allergenicity of MON 89034 x 1507 x MON 88017 x 59122 and its transgenic proteins, in line with its guidance and the internationally harmonized approach as described in *Codex alimentarius* guidelines. This weight of evidence also includes, besides the outcomes of the updated bioinformatics-supported comparisons and the issues previously considered in the evaluations of the single parental events (MON 89034, 1507, MON 88017, 59122), including the history of allergenicity, if any, of the sources of the transgenic proteins and the *in vitro* resistance of the transgenic proteins towards proteolytic enzymes. Also the potential unintended change in intrinsic allergenicity of the host maize has been considered in these opinions. The EFSA GMO Panel is of the opinion that discussion on this issue should be closed. Cry proteins have been already assessed by the GMO Panel. In previous opinions, the EFSA GMO

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			thuringiensis. Biochem Biophys Res Commun., 62: 517-521 Rojas-Hernández S, Rodríguez-Monroy MA, López-Revilla R, Reséndiz-Albor AA, Moreno-Fierros L., 2004. Intranasal coadministration of the Cry1Ac protoxin with amoebal lysates increases protection against Naegleria fowleri meningoencephalitis. Infect Immun., 72:4368-4375 Vazquez-Padron RI. Martinez-Gil AF. Ayra-Pardo C. Gonzalez-Cabrera J. Prieto-Samsonov DL. de la Riva GA., 1998. Biochemical characterization of the third domain from Bacillus thuringiensis Cry1A toxins. Biochem Mol Biol Int., 45(5):1011-20. Vazquez RI. Moreno-Fierros L. Neri-Bazan L. De La Riva GA. Lopez-Revilla R., 1999. Bacillus thuringensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol., 49: 578-84. Vazquez-Padron RI. Gonzales-Cabrera J. Garcia-Tovar C. Neri-Bazan L. Lopez-Revilla R. Hernandez M. Moreno-Fierro L. de la Riva GA., 2000a. Cry1Ac protoxin from Bacillus thuringiensis sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. Biochem Biophys Res Commun., 271:54-8 Vazquez-Padron RI. Moreno-Fierros L. Neri-Bazan L. Martinez-Gil AF. de-la-Riva GA. Lopez-Revilla R., 2000b. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz J Med Biol Res., 33: 147-55.	Panel assessed the allerginicity of Cry proteins and the allergenicity of the whole GM plant (i.e. 59122 maize), and took into consideration the potentional adjuvanticity of Cry proteins that is mentioned in the comment. The EFSA GMO Panel confirms its previous opinion and still considers that since maize is not a common allergenic food, even if the presence of a newly expressed Cry protein might enhance an immune response to endogenous maize protein(s), it is very unlikely that this would modify the allergenicity of the whole GM crop.
Spain	Ministry of the Environment, and Rural and Marine Affairs	A. General information	The Spanish National Commission on Biosafety considers that this dossier should be a stand-alone document including all relevant information on the different events present in the final GMO. Furthermore, the notification should contain all the necessary information for assessing the hybrid.	The application is in line with the EFSA guidance document (2007).
Spain	Ministry of the Environment, and Rural and Marine Affairs	C. Information relating to the genetic modification	The dossier does not include the chromosome location of the different insertions have happened. This data is relevant to exclude potential interactions of the transgenes into the genome.	Information on the chromosomal location of the insert(s) is not considered necessary to carry out the risk assessment.

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Spain	Ministry of the Environment, and Rural and Marine Affairs	D, 07 Information on any toxic, allergenic or other harmful effects on human or	Toxicological and allergenic studies should be provided with the expressed proteins in a combined way into the hybrid. Only repeat dose studies about two proteins have been submitted; the rest of the investigations only include one dose tests.	The safety of the newly expressed proteins was previously evaluated by the EFSA GMO Panel in its opinions on the single parental events for this stacked event (MON 89034, 1507, MON 88017, 59122). Items considered for the safety of these proteins included *in vivo* toxicity testing with the purified protein (including 28-days study with the Cry34Ab1/Cry35Ab proteins that are also expressed in maize 59122, provided by the applicants at the EFSA GMO Panel's request, *in vitro* resistance to proteolytic degradation, bioinformatics-supported comparisons of the amino acid sequences of the newly expressed proteins with known toxins), and other characteristics of the proteins (e.g. glycosylation). At the request of the EFSA GMO Panel. The applicants provided a risk assessment of potential interactions among the single events with regard to human and animal health, in its response dated 23 June 2009. The EFSA GMO Panel concludes in its opinion, section 5.1.4.1, that "*Determination of the levels of the newly expressed proteins in grain produced by maize MON 89034 x 1507 x MON 88017 x 59122 showed comparable levels to those in the respective single maize events (see section 3.1.4). On the basis of the known functions and modes of action, the EFSA GMO Panel considers it unlikely that interactions between these newly expressed proteins (Cry1A.105, Cry2Ab2, Cry1F, PAT, Cry3Bb1, CP4 EPSPS, Cry34Ab1, and Cry35Ab1) would occur that would raise any safety concern.*"

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