| Application EFSA-GMO-UK-2005-15 (Maize 1507x59122) ANNEX G<br>Comments and opinions submitted by Member States during the three-month consultation period | | | | |
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| **Country** | **Organisation** | **Reference** | **Comment** | **EFSA GMO Panel response** |
| **Comments from National Competent Authorities under Directive 2001/18/EC** | | | | |
| Austria | Ministry of Health, Family and Youth | General comments | Concerning the single events of this notification, Austria is still of the opinion that their risk assessment with regard to e.g. molecular characterisation, allergological and toxicological as well as environmental risk assessment can not be regarded as sufficient. Due to these lacks in the presented scientific data of the single events, it is not regarded as appropriate to apply for approval of the stacked event before clarifying the shortcomings of the single events. | The EFSA GMO Panel adopted scientific opinions on applications for placing on the market of genetically modified maize containing the single events which are stacked in 1507x59122 maize.<br>Maize 59122 was authorised under Regulation (EC) No 1829/2003 with Commission Decision 2007/702/EC (EC, 2007).<br>Maize 1507 was authorised under Directive 2001/18/EC by Commission Decision 2005/772/EC (EC, 2005b) for feed use, import and processing. The placing of 1507 maize on the market for food use received authorisation under Regulation 1829/2003 with Commission Decision 2006/197/EC (EC, 2006). |
| Austria | Ministry of Health, Family and Youth | General comments | Detection method As long as no official (guidance) document on the interpretation of detection results of the described method for stacked events are available, no approval for placing on the market of this product should be given. | Not in the remit of the Panel. |
| Austria | Ministry of Health, Family and Youth | C. Information relating to the genetic modification | The data submitted to conclude the molecular equivalence of GM Maize 1507x59122 with the parental GM lines (59122 and 1507) consist of Southern Blots to demonstrate presence of the introduced traits (Cry34Ab1, Cry35Ab1, Cry1F, and Pat) in GM Maize 1507x59122. However the used probes do not span the complete inserts introduced into the parental GM plants used to construct GM Maize 1507x59122. They only represent parts of the coding regions of the introduced genes. The rather limited scope of analysis as presented does not result in a comprehensive examination of the inserts present in GM Maize 1507x59122. Some of the data are furthermore not fully conclusive. • Some parts of the analysis, e.g. the demonstration of the molecular identity of the 59122 trait of GM Maize 1507x59122 are limited to internal insert sequences and do not cover both border regions of the insert. For a complete molecular characterisation and the | Additional information has been requested on the intactness of the inserts and the flanks.<br>Molecular equivalence of the 1507 and 59122 insert in the hybrid line was determined by Southern analysis, using SacI and HindIII digested genomic DNA and probes of the pat, cry34Ab1, cry35Ab1 and cry1F genes. From the hybridisation patters of 1507 x 59122 maize and both parental lines it was concluded that the organisation of sequences in the insert is unchanged. Also the intactness of the 1507 insert and of the 3' side of the 59122 was confirmed.<br>Additional information has been supplied on the intactness of the 5' of the 59122 insert in the hybrid line. The intactness of the 59122 insert in the hybrid line was confirmed by results obtained by event-specific real time PCR of the 5' region of the 59122 insert.<br><br>Differences in migration observed in fig 5, p. 60 between hybridising *cry*1F-containing fragments of |

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| | | | comparison with parental GMO-strains, results for insert and border regions of the introduced traits should be presented by the notifier. • Some assumptions by the notifier do not seem to be justified due to bad quality of data (e.g. Fig. 5, p. 60: hybridising fragments of plasmid PHP8999 cannot be regarded to migrate equivalently by the figure presented in the application). The method used for all Southern experiments employed Digoxygenin-labelled probes and DNA Molecular Weight Markers (MWM). As noted in the application these DIG-labelled MWM typically migrate slower in Agarose gels than expected from their molecular weight by a margin of 5-10%. This makes the necessary comparisons difficult and adds to the methodological difficulties of a method like Southern blotting. The notifier therefore has to assume that the identified fragments are of the expected size rather than unequivocally demonstrating it. We therefore suggest that methods are used for molecular characterisation, which do not introduce avoidable uncertainties. For a detailed characterisation of modifications present in GM Maize 1507x59122 the notifier makes reference to the data submitted for parental GM events. However since the demonstration of molecular identity lacks strength due to the indicated inconsistencies, we do not regard the conclusions of the notifier justified. | 1507 x 59122, 1507 and *cry*1F containing PHP8999 are due to differences in DNA concentration. Differences in migration rate due to concentration differences are a commonly observed phenomenon in gel electrophoresis<br><br>The DIG labelled MW markers migrate slower in agarose gel. However since hybridizing fragments migrate equivalently with the hybridizing bands of the plasmid controls, the GMO panel is of the opinion that the size of the fragments is sufficiently demonstrated.<br><br>For the analyses of the integrity of the inserts in the stack, the only meaningful comparators are the single events. |
| Austria | Ministry of Health, Family and Youth | C. Information relating to the genetic modification | The referenced data for parental GM events itself were criticised in our comments addressed to the respective applications. The mentioned concerns still prevail because similar data are submitted in the application for GM Maize 1507x59122: Concerning GM Maize 59122: Regarding characterisation of the maize genomic regions at the border regions 5´and 3´of the transgenic insert the annexed laboratory study report (Annex 7 of the technical dossier for GM Maize 59122) states that “No further identification of the maize genomic border sequences was possible due to limited sequence homology with publicly | An updated bioinformatic analysis has been requested on of both events.<br>For 59122 an updated BLAST analysis indicated that the DNA in 59122 was inserted 1032 bp downstream of the coding region of a maize pentatricopeptide repeat (PPR) protein, the empty pericarp4 (*emp4*). This PPR protein is essential for seed development in maize. In event 59122 seed development is not affected suggesting that expression of *emp4* was not altered by the insertion.<br>For 1507 an updated BLAST analysis of the flanking DNA sequences suggests that the insert in 1507 is |

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| | | | available sequences in GenBank." (Annex 7, p.3). This conclusion was drawn upon a homology search against sequences contained in GenBank Rel. 138 (Oct. 25th 2003). It is notable however that since the time this study was undertaken the number of DNA sequences stored in GenBank significantly increased (for a graphic representation see: http://www.ncbi.nlm.nih.gov/Genbank/index.html). It is therefore necessary to compare the identified border sequences in GM maize 59122 against a current version of the database to aid better identification of the genomic region into which the transgenic DNA was inserted. Therefore the submitted information is incomplete. Concerning GM Maize 1507: Incompleteness of the molecular characterisation was criticised for various applications of GM Maize 1507, like the referenced application (EFSA-GMO-NL-2004-02), specifically concerning location and size of the additional copy of cry1F in the GM Maize 1507 genome. Further concerns address the question whether this additional copy of the cry1F gene in the GM event 1507 contains an ubiquitin promoter region. | flanked by a putative RIRE2 retrotransposon (downstream) and a Huck1 retrotransposable element (upstream).<br><br>For 1507 the updated bioinformatic analysis confirmed the location of the additional copy of the *cry* gene in the insert. Analysis of novel ORFs that have the potential to be transcribed, do not give rise to proteins that have significant homology to know toxins or allergens. Therefore the GMO panel concludes there is no safety concern. |
| Austria | Ministry of Health, Family and Youth | D, 03 Information on the expression of the insert | Information on the expression of CRY/PAT/EPSPS proteins The control used (36B08 isoline to GM Maize 1507x59122) is described as a Pioneer commercial hybrid with a background that is representative of GM Maize 1507x59122. However no breeding history is supplied to assess whether the strain is derived from a genetically modified strain, as is apparently the case with the control for another related Stacked Event GM Maize 1507x59122xNK603. According to EFSA guidance non-GMO controls should not be derivatives of genetically modified lines. We therefore request submission of further information on the control strain. The controls used furthermore do not meet the requirements of the newly published EFSA "Guidance Document of the Scientific Panel on GMOs for the risk assessment of genetically modified plants containing stacked transformation events" [EFSA | Additional information has been requested on the controls used for protein expression and was supplied by the applicant.<br><br>The applicant provided new data from European field trials with 1507 x 59122 in 2005, on three locations in Spain. In these trials both parental lines 1507 and 59122 were used as controls. Results on expression levels of the three Cry proteins and the PAT protein demonstrated levels in the hybrid line to be in the same range as in the parental lines. No effect was apparent for herbicide application. The GMO panel considers these data on expression of the insert sufficient for the safety assessment.<br><br>Maize 1507x59122 does not contain the CP4 EPSPS protein. Upon request by the GMO Panel, the applicant |

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| | | | (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.], calling for testing of a Non-GMO control strain of comparable genetic background together with the parental GM-events and the Stacked Event. The field trial were conducted 2003 in the USA (3 locations) and Ca (2 locations), comparing sprayed (2x glufosinate ammonium) and unsprayed stacked event 1507x59122 with a non-GM control. Plant protection included the application of metalochlor, atrazine, chlorpyrifos, dimethanamid, terbufos, benoxacor, permethrin, dicamba and tefluthrin. Two of the control root samples were contaminated with traceable Cry34Ab1 protein (0,08 and 0,7ng/mg). Expression of Cry35Ab1 was detected in 3 control tissue samples (0,08, 0,09, 0,07ng/mg). Thus the conclusion "All control samples were negative for the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins" is not correct (page 36, Annex 2). Therefore it can be stated that theses contaminations can not be regarded as state of the art. Furthermore data on expression of transgenic proteins are submitted in the technical dossier as pooled values over all locations (5 sites in USA and Canada). The results show that the expression levels vary considerably across locations analysed, with standard deviations consistently at about 35% of the mean values, for PAT at more than 100% of mean values. We therefore regard the analysis as incomplete and insufficient. In our opinion appropriate controls need to be used and additional data from parental GM Maize lines 59122 and 1507 should be submitted. These data should be analysed and presented for individual sites and across sites. | provided detailed information on the genetic background of the non-GM control used in the study on agronomic characteristics, quantitative ELISA and nutrient composition analysis of maize 1507x59122 (Annex 2). The additional information including a breeding history confirmed that the control was **not** derived from a genetically modified strain.<br><br>Furthermore, the applicant provided a study that measured the protein concentrations of Cry34Ab1, Cry35Ab1, Cry1F, and/or PAT in tissues sampled from 1507, 59122, and 1507x59122 maize. The field phase of this study was conducted in Spain and contained three separate field locations. Each location contained maize 1507, 59122 and 1507x59122. Plots of maize 1507, 59122 and 1507x59122 were untreated or treated with two applications of a herbicide containing glufosinate. Throughout the growing season leaf, root, whole plant, pollen, stalk, forage, and grain samples were collected for protein concentration analysis. With regard to comparative agronomic and compositional analysis, the Panel considered the data for maize 59122 and maize 1507 provided with the applications for the single events. Individual values for individual locations concerning expression levels, agronomic and compositional data can be retrieved from the Annexes of the applications. Overall, the Panel found the set of studies on expression levels, as well as on agronomic and compositional characteristics to be in-line with the Guidance documents and sufficient to conclude on equivalence to the non-GM comparator and on the safety of the newly expressed proteins. |
| Austria | Ministry of Health, Family and | D, 03 Information on the expression of the insert | Expression of potential fusion proteins No updated analysis on expression of potential fusion proteins was submitted for GM Maize 1507x59122. The in silico analyses for parental GM events GM Maize | An updated bioinformatic analysis has been requested for both events and was supplied by the applicant. This analysis confirms earlier safety assessments of both events. |

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| | Youth | | 59122 and GM Maize 1507 are furthermore not supported by experimental data to assess which sequences coding for potential fusion proteins are actually transcribed/translated in GM maize 1507x59122. Such data are necessary to substantiate the conclusions of the notifier. | Bioinformatic analyses indicate that should any of the transcripts be translated, none of the potential peptides would show homology with known allergens, toxins or other biologically active proteins or peptides. |
| Austria | Ministry of Health, Family and Youth | D, 04 Information on how the GM plant differs from the recipient plant in: … | The control used (36B08 isoline to GM Maize 1507x59122) is described as a Pioneer commercial hybrid with a background that is representative of GM Maize 1507x59122. However no breeding history is supplied to assess whether the strain is derived from a genetically modified strain, as is apparently the case with the control for another related Stacked Event GM Maize 1507x59122xNK603. According to EFSA guidance non-GMO controls should not be derivatives of genetically modified lines. We therefore request submission of further information on the control strain. Data are presented in the technical dossier as values across sites (see technical dossier p. 71, Table 5). Some significant differences are indicated for the results, specifically for plant height, early and final population counts, but were not followed up by the notifier. Furthermore parameters “insect damage” and “disease incidence” were evaluated only semi-quantitatively and the notifier did not differentiate between individual insect pest species or diseases. Such an assessment can only indicate rough differences in the susceptibility of a plant to certain stressors. Also certain differences in the susceptibility to specific insects (e.g. secondary pests) or diseases cannot be detected by such an analysis. More specific data are requested to justify the conclusions by the notifier. Additionally the notifier should present an analysis of single site results for agronomic parameters. The agronomic traits were only compared between the non-spayed 1507x59122 GM maize and its control to evaluate the potential impact of the GM. Significant differences for the stacked event 1507x59122 only concerned higher mean plant height. There was no significant | Additional information has been requested on the controls used for protein expression and was supplied by the applicant.<br><br>The applicant provided new data from European field trials with 1507 x 59122 in 2005, on three locations in Spain. In these trials both parental lines 1507 and 59122 were used as controls. Results on expression levels of the three Cry proteins and the PAT protein demonstrated levels in the hybrid line to be in the same range as in the parental lines. No effect was apparent for herbicide application. The GMO panel considers these data on expression of the insert sufficient for the safety assessment.<br><br>The 59122 maize, used in the cross with maize 1507 to produce maize 1507 x 59122, was hybrid seed from a first backcross generation (BC1 hybrid) and it was obtained as follows. The original transformant, containing the DAS-59122-7 insert, was crossed with inbred 09B resulting in the T1 generation. The T1 generation was then crossed with the inbred line 05F followed by two subsequent crossings with line 1W2 resulting in the first backcross generation (BC1 generation). The maize 59122BC1 generation was then crossed with maize 1507 fixed into POZ3 elite inbred line to produce 1507 x 59122 F1 hybrid. The non-GM control maize for this study was obtained by means of a single cross between inbreds POZ3 and 1W2.<br><br>The GMO Panel considers that these controls are appropriate.<br><br>The scope of the application is for import, processing as |

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| | | | difference concerning insect damage described as the level of destructive insect resistance at maturity. It is thus not clear, where the perceived advantages of the insect-resistant 1507x59122 GM maize line is becoming manifest. Were the target pests of the recombinant toxins not present or is the performance of the non-GM control just as good? This point should be clarified. | well as for food and feed uses and does not include cultivation. Therefore, there was no requirement for scientific information on possible environmental effects associated with the cultivation.<br><br>With regard to significant differences for agronomic data, see text in the opinion. The Panel does not see an impact on safety arising from the data on insect resistance for maize 1507x59122 compared to the non-GM control. |
| Austria | Ministry of Health, Family and Youth | D, 07.01 Comparative assessment | The control used (36B08 isoline to GM Maize 1507x59122) is described as a Pioneer commercial hybrid with a background that is representative of GM Maize 1507x59122. However no breeding history is supplied to assess whether the strain is derived from a genetically modified strain, as is apparently the case with the control for another related Stacked Event GM Maize 1507x59122xNK603. According to EFSA guidance non-GMO controls should not be derivatives of genetically modified lines. We therefore request submission of further information on the control strain. The controls used furthermore do not meet the requirements of the newly published EFSA "Guidance Document of the Scientific Panel on GMOs for the risk assessment of genetically modified plants containing stacked transformation events" [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.], calling for testing of a Non-GMO control strain of comparable genetic background together with the parental GM-events and the Stacked Event. The results of the compositional analyses according to OECD guidelines show significant differences for a number of analytes across locations (see technical dossier p. 27 ff, and Annex 2): 11 significant differences across locations were found for comparisons of GM Maize 1507x59122 differently treated with Glufosinate or without Glufosinate, | Additional information has been requested on the controls used for protein expression and was supplied by the applicant.<br><br>The applicant provided new data from European field trials with 1507 x 59122 in 2005, on three locations in Spain. In these trials both parental lines 1507 and 59122 were used as controls. Results on expression levels of the three Cry proteins and the PAT protein demonstrated levels in the hybrid line to be in the same range as in the parental lines. No effect was apparent for herbicide application. The GMO panel considers these data on expression of the insert sufficient for the safety assessment.<br><br>The maize 59122, used in the cross with maize 1507 to produce maize 1507 x 59122 , was hybrid seed from a first backcross generation (BC1 hybrid) and it was obtained as follows. The original transformant, containing the DAS-59122-7 insert, was crossed with inbred 09B resulting in the T1 generation. The T1 generation was then crossed with the inbred line 05F followed by two subsequent crossings with line 1W2 resulting in the first backcross generation (BC1 generation). The maize 59122 BC1 generation was then crossed with maize 1507 fixed into POZ3 elite inbred line to produce 1507 x 59122 F1 hybrid. The non-GM control maize for this study was obtained by means of a single cross between inbreds POZ3 and 1W2. |

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| | | | respectively and the control for all of the categories of compounds assessed. A few analytes furthermore showed significant differences at a majority of individual locations. For the result for mean ash values a contradictory description (with 2 conflicting pieces of information) is presented (technical dossier p. 27: for GM Maize 1507x59122 untreated, Proximates and Fiber Analysis). This should be corrected. Based on comparison with results from other trials as reported in literature, no further evidence to assess the reasons for the differences was submitted. The observed differences in a majority of compounds analysed should gain more attention to clarify the underlying causes. For compounds, which do not show statistically significant differences across locations no results for individual sites are analysed in the technical dossier. Therefore we request an additional analysis showing which site related significant differences were found. The statement in the Technical Dossier, Part 1, that "Maize is not considered to have any harmful effects on human or animal health (Del Valle 1983)" has to be revised. Two endocrine-disrupting agents, tetrahydrofuran-diol and leukotoxin-diol derivatives of linoleic acid, have been discovered in some maize lines, causing reproductive disorders in rodents at extremely low levels, about 200 times lower than other phytoestrogens (Markaverich et al. 2002, 2005[Markaverich et al (2002). "A Novel Endocrine-Disrupting Agent in Corn with Mitogenic Activity in Human Breast and Prostatic Cancer Cells," Environmental Health Perspectives, 110(2), Feb. 2002, pp. 169-177 Markaverich et al (2005). "Leukotoxin Diols from Ground Corncob Bedding Disrupt Estrous Cyclicity in Rats and Stimulate MCF-7 Breast Cancer Cell Proliferation," Environmental Health Perspectives, 113(12), Dec. 2005, pp. 1698-1704; ] ). The analyses of the stacked event 1507x59122 do not include these compounds. Unfortunately there is also no information included in | The GMO Panel considers that these controls are appropriate.<br>With regard to significant compositional differences: see text in the opinion.<br>Despite the contradictory assessment of ash values given in the Technical Dossier, the Panel considered the data as set-out in Annex 2. This resulted in the conclusion that mean ash values across locations are statistically significantly different for untreated 1507x59122 maize compared to the non-GM control. However, no statistically significant differences for ash mean values were observed at any of the five individual locations.<br>Considering the fact, that compositional equivalence between maize 1507x59122 and the non-GM control was established, the Panel does not require further assessment of compositional differences observed at individual locations.<br>The spectrum of compounds analysed is in compliance with the respective OECD Consensus document and with the EFSA GMO Panel Guidance Document. |

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| | | | the technical dossier that the germlines used for their production do not express the newly discovered toxins. | |
| Austria | Ministry of Health, Family and Youth | D, 07.01 Comparative assessment | In previous opinions (stacked events 59122xNK603 and 1507x59122xNK603 GM maize lines) consistent significant differences concerning the C/N ratio were found.. Comparing the carbohydrate and crude protein values between the stacked event 1507x59122 and its non-GM control similar differences were not found, except for 2 locations in Canada (ON1 and ON2). The comparisons concern the same parental lines and derived stacked lines this is surprising. The possibility of a location x weather x genotype-interaction could possibly offer an explanation. But the GM maize lines 59122, 59122xNK603, 1507x59122xNK603 and the here compared 1507x59122 were all grown in 2003 in the same regions: 3 locations in the USA and 2 in Canada. The table of monthly temperatures and rainfall show, that 2003 was much drier as compared to the 10 year average precipitation in the region. Since the mineral N uptake is strongly influenced by weather conditions no final conclusion can be drawn from one season only, especially if the season is not near the 10 year average rainfall. Data from more than one single growing season should thus be submitted by the notifier. Data from literature additionally to and other than OECD documents were used to calculate a range for additional comparisons and in case significant differences between the GM and the non-GM maize were observed. These data are derived from different sources and from field trials other than those carried out specifically with GM Maize 1507x59122 and the control line. This approach results in data derived from maize plants grown under different conditions and in different years and introduces additional variation which may obscure relevant differences. Based on such data the notifier concludes that GM Maize 1507x59122 is substantially equivalent to non-modified maize in | The EFSA GMO Panel has recently adopted a scientific opinion on maize 1507x59122xNK603 and did not identify any safety concern (EFSA, 2009).<br><br>59122xNK603 maize was approved (EFSA, 2008) and its compositional equivalence with the non-GM comparator was accepted by the GMO Panel based on all the information provided (see opinion).<br>In the present application, since compositional data derived from one season did not reveal consistent differences between maize 1507x59122 and the non-GM control, the Panel does not see a need for an additional field trial covering a second growing season. The approach followed by the applicant to assess the compositional data and to conclude on compositional equivalence is accepted by the GMO Panel. |

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| | | | spite of differences observed across locations and for individual locations. It is unclear, which significant differences would actually trigger further investigations. The assessment of compositional equivalence between the GM and the non-GM plant is not considered to be a safety assessment in itself, but rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart (Codex Alimentarius Commission 2003 )[Codex Alimentarius Commission (2003). Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45.]. | |
| Austria | Ministry of Health, Family and Youth | D, 07.04 Agronomic traits | The control used (36B08 isoline to GM Maize 1507x59122) is described as a Pioneer commercial hybrid with a background that is representative of GM Maize 1507x59122. However no breeding history is supplied to assess whether the strain is derived from a genetically modified strain, as is apparently the case with the control for another related Stacked Event GM Maize 1507x59122xNK603. According to EFSA guidance non-GMO controls should not be derivatives of genetically modified lines. We therefore request submission of further information on the control strain. Agronomic characteristics were presented in the technical dossier as values across sites (technical dossier p. 71, table 5). A calculation across sites masks differences at single sites due to regionally different frequencies of pest or pathogen infestations. The individual parameters should therefore also be analysed on a single location basis for all data (USA/Canada). | Analysis of individual parameters on a single location basis is given in Annex 2. |
| Austria | Ministry of Health, Family and Youth | D, 07.08 Toxicology | For the whole food/feed safety assessments the applicant refers to the testing of whole grains in a feeding study with poultry (42 day study). The notifier states that for comparison together with GM Maize 1507x59122 grain from a near isoline control and three commercial non-GM lines have been tested (technical dossier p. 36). Since the referenced Annex (Annex 3) only describes tests with two non-GM lines | It is correct that according to Annex 3, two reference control maize lines were used in the 42-day poultry study in addition to the non-GM comparator.<br>The panel agrees that such a study would rather pertain to nutritional than to toxicological assessment. It also considers that the present one is of lmited value (se opinion).<br>However the panel emphasizes that since maize |

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| | | | the statement needs to be corrected. The referenced study itself does constitute a feed conversion study rather than a toxicological study. For safety considerations toxicological endpoints must be assessed rather than performance parameters as done in the chicken study supplied. Such a feeding study with chicken broilers is therefore not appropriate to assess the toxicological safety of GM Maize 1507x59122. Relevant toxicological data established for GM Maize 1507x59122 as required by the newly published EFSA guidance for stacked events [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.] should be submitted. | 1507x59122 is agronomically and compositionally equivalent to its non-GM control, no toxicological studies with laboratory animals nor nutritional studies are required. |
| Austria | Ministry of Health, Family and Youth | D, 07.08 Toxicology | The notifier argues that Bacillus thuringiensis derived proteins (Cry34Ab1, Cry35Ab1, Cry1F) have a history of safe use. However since the introduced traits are not originating from a commonly used food source a safe history of consumption may not be deduced. For safe history of use see comments to chapter 7.9 (Allergenicity). For the toxicological assessment of GM Maize 1507x59122 the applicant refers to the assessment of the individual gene products with reference to acute toxicity studies of microbially produced test proteins among others. However some proteins (Cry35Ab1 produced by the P. fluorescens strain MR1256) show minor differences to Cry35Ab1 protein as expressed in GM Maize 1507x59122. Tests employing heterologous test proteins should be done with similar test material to obtain conclusive results. Additionally, the safety of the proteins is concluded with reference to digestion patterns of the individual proteins in simulated gastric fluids. The respective proteins were tested separately in these experiments, wheras in vivo the proteins are both present in the digestive system. Therefore the employed experimental setup does not reflect the real exposition scenario of consumption of these proteins | The GMO Panel agrees with the approach followed by the applicant with regard to the safety assessment of newly expressed proteins. It is justified to refer to the safety assessment of the individual gene products as provided with the applications on the single events. Concerning a potential interaction of the newly expressed proteins in the stacked event, the Panel considered it unlikely based on the overall information provided (see opinion). |

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| | | | in GM Maize 1507x59122. Generally, little significance can be attributed to toxicological tests with isolated gene products. This has already been mentioned by many authors (Spök A., Hofer H., Lehner P., Valenta R., Stirn S. Gaugitsch H. (2005). Risk Assessment of GMO Products in the European Union. Umweltbundesamt Wien, Band 253. Millstone E. (1999), Beyond substantial equivalence. Nature 401 (6753): 525-526 Walker R. (2000). Joint FAO/WHO Expert consultation on foods derived from Biotechnology. 29 May-2 June 2000. Geneva. ) due to the fact that pleiotropic effects in the plant as well as differences in protein quality remain unconsidered. There is scientific evidence that the parameters studied do not necessarily prove the toxicological or allergological safety of proteins (see references in Spök et al., 2005). No data on potential interactions of introduced traits with relevance to adverse effects are given. Such an assessment is crucial for an assessment of Stacked Event GMOs according to published guidelines [EFSA (2007), Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, EFSA Journal 512, 1-5.] and therefore requested. | |
| Austria | Ministry of Health, Family and Youth | D, 07.08 Toxicology | Concerning statistics the concurrent control data are more appropriate than historical reference ranges for comparison with test material treatment group (Weingand et al. 1990 )[Weingand, K., Brown, G., Hall, R., Davies, D., Gossett, K. , Neptung, D. et al. Harmonization of Animal Clinical Pathology Testing in Toxicity and Safety Studies: Toxicological Sciences 2002, v. 29, p. 198-201.]. The data were analyzed using PROC MIXED procedure of SAS. The MIXED procedure fits a variety of mixed linear models to data and enables to use these fitted models to make statistical inferences about the data. Additionally data were evaluated using parametric tests highly recommended in toxicology studies (Festing & | Referenced study unclear. In the study provided with Annex III, no separate analysis for males and females is provided, however, data given for body weights in the MS comment are reference ranges.<br>Concerning the need for toxicity/nutritional studies involving laboratory animals: see above. |

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| | | | Altmann, 2002 )[Festing, M. F. W. & Altman, D. G. Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR Journal, 2002, 43, 244-258]. It is not clear whether data were statistically evaluated separately for different sexes . However average body weight of males differed markedly from females (2088g vs 1741g). Additionally it has to mentioned that although the risk of synergistic effects in stacked events are mentioned in the EFSA Guidance Document for stacked events, no whole feed toxicity studies have been conducted. Generally toxicological risk assessment should be performed following the OECD test guidelines for subchronic studies No407 (28-day oral toxicity study in rodents), 408 (90-day oral toxicity study in rodents) and No 409 (90-day oral toxicity study in non-rodents). Furthermore for chronic evaluation carcinogenicity studies could be performed (No 451) to evaluate potential mutagenicity hazard. This design is also suggested by the SAFOTEST working group following a two step safety procedure of in vitro and in vivo investigation (Poulsen et al., 2007 )[Poulsen, M.; Kroghsbo, S.; Schroder, M.; Wilcks, A.; Jacobsen, H.; Miller, A.; Frenzel, T.; Danier, J.; Rychlik, M.; Shu, Q.; Emami, K.; Sudhakar, D.; Gatehouse, A.; Engel, K. H.; Knudsen, I. A 90-Day Safety Study in Wistar Rats Fed Genetically Modified Rice Expressing Snowdrop Lectin Galanthus Nivalis (GNA). Food and Chemical Toxicology 2007, 45, 350-363] Only whole food/feed studies such as feed conversion studies with farm animals and 90-day studies with rodents reflect realistic conditions. But no general statements about potential adverse effects on the long run are possible. Organisms generally have the capacity to bear up with a relatively short-time exposure to inadequate feed. Isogenetic animal strains should be considered because they are usually more uniform phenotypically than commonly used outbred stocks. They can be more powerful to detect smaller treatment responses. When it is necessary to | |

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| | | | replicate an experiment across a range of possible susceptibility phenotypes, small numbers of animals of several different inbred strains can be used in a factorial experimental design without any substantial increase in total. The advantage of this design is that the importance of genetic variation in response can be quantified (Festing & Altmann, 2002). For additional comments see chapter "Nutritional Assessment". | |
| Austria | Ministry of Health, Family and Youth | D, 07.09 Allergenicity | Assessment of allergenicity of the whole GM plant or crop With regard to the assessment of allergenicity of the whole GM plant the notifier concludes that there is no difference to other conventional maize. However the notifier states that maize allergies mainly caused by pollen do exist, albeit in rare cases. The Cry proteins present in GM Maize 1507x59122 are expressed in pollen as shown by the notifier. References cited above suggest that Cry proteins might have an allergenic potential, specifically with incorporation by ways other than ingestion, e.g. contact in the respiratory tract. The notifier does not consider the allergenic potential of low level expression of Cry proteins in pollen of maize cultures contaminated with GM Maize 1507x59122. We request that consideration is paid to this alternative route for sensibilisation against and allergenicity of the transgenic Cry proteins for any risk assessment of GM Maize 1507x59122 with regard to allergenicity. | Single events and newly expressed protein in single events, particularly Cry proteins) have been already assessed, including for allergenicity.<br>The Panel is not aware of any new information that would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between these newly expressed proteins that would in particular impact on the allergenicity.<br>With regards the allergenicity of the whole plant, the panel is aware of the rare cases of allergy to maize, which however is not considered a common allergenic food. The Panel sees no reason to consider that the allergenicity of the GM maize (e.g. the single events already assessed as well as the present stack event) would be changed because of the genetic modification. |
| Austria | Ministry of Health, Family and Youth | D, 07.09 Allergenicity | The relevance of some of these parameters is questionable and not considered to be of indicative value with regard to safety: • Since no threshold levels for sensitisation to potential allergens can be established, the criterion that introduced proteins are expressed at lower levels than most common food allergens is not conclusive. Source materials are qualified as non-allergenic by the notifier. However this conclusion cannot be justified with a view to data suggesting an allergenic potential at least for Cry proteins [Bernstein L.I., Bernstein J.A., Miller M., Tierzieva S., Bernstein D.I. Lummus Z., Selgrade | Single events and newly expressed protein in single events, particularly Cry proteins) have been already assessed, including for allergenicity.<br>The Panel is not aware of any new information that would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between these newly expressed proteins that would in particular impact on the allergenicity.<br>With regards the allergenicity of the whole plant, the panel is aware of the rare cases of allergy to maize, which however is not considered a common allergenic |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | J.K., Doerfler D.L., Seligy V.L. (1999), Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides.Environ. Health Perspectives 107(7): 575-582; Doekes G., Larsen P., Sigsgaard T., Baelum J. (2004), IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: the BIOGART study. Am J Ind Med. 46(4):404-7. ]. The question whether due to the insertion new allergenic proteins might be expressed by the GM plant is not regarded relevant for maize, since maize is not a major allergenic food, but the source of the inserted gene also has to be considered. Bernstein et al. (1999) investigated immune responses occurring in farm workers exposed to Bt containing pesticides and found indications that exposure to Bt sprays may lead to allergic skin sensitization and induction of IgE and IgG antibodies, or both. It may now be possible to test for Bt toxins. If a gene product is derived from a source with kown allergenic potential, there is a reasonable certainty that the GM crop will be allergenic (D´Mello 2003 )[D´Mello, J.P.F. (2003): Food Safety: Contaminants and Toxins. CABI Publishing; p.367]. But the reverse does not guarantee safety either. Of course it would be complicated to follow up on direct allergenicity testing if along the decision tree the aswer as to whether the source is allergenic or not is “yes”, since test materials and previously exposed humans are not easily available. Corroborating the assumption that Bt toxins might exhibit allergenic potential are the findings about the Cry1C and the Cry1Ac toxins (Vasquez Padron et al. 1999, 2000 a+b). Furthermore recent reports from India in connection with allergic reactions in workers handling Bt cotton (Source: Frontline 23(12), India, by Venkitesh Ramakrishnan http://www.hinduonnet.com/fline/stories/20060630004102200.htm date: 17-30 Jun 2006) and additionally reports on a possible connection between | food. The Panel sees no reason to consider that the allergenicity of the GM maize (e.g. the single events already assessed as well as the present stack event) would be changed because of the genetic modification. |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | the inhalation of Bt maize pollen and adverse effects in Philippine villages have been published (Traavik & Smith, 2004 )[Terje Traavik & Jeffrey Smith (2004): Bt-maize (corn) during pollination, may trigger disease in people living near the cornfield. http://www.mindfully.org/GE/2004/Bt-Corn-Human-Disease24feb04.htm]. Although these last mentioned reports don't present scientific papers, it could at least be expected that detailed investigations on a scientific basis are conducted to follow up these indications. But so far these observations and results have not been included in any discussions or assumptions about the allergenicity of Bt toxins In the Cry1F insert a single 6 amino acid match with the Der p7 protein of dust mite (Dermatophagoides pterimyssinus) was identified. But no evidence of cross-reactivity between Cry1F and Der p7 was observed in the serum of sensitive test persons (Ladics et al. 2006 )[Ladics, S.G., Bardina, L., Cressman, R.F., Mattsson, J.L., Sampson, H.A. (2006): Lack of cross-reactivity between Bacillus thurengiensis derived protein Cry1F in maize and dust mite Der p7 protein with human sera positive for Der p7-IgE. Regulatory Toxicology and Pharmacology, Vol 44, Issue 2; pp.136-143 ]. | |
| Austria | Ministry of Health, Family and Youth | D, 07.09 Allergenicity | • Digestion experiments in simulated gastric environments for introduced proteins are of limited significance with regard to the methods used [See for instance: Fu, T.J. (2002), Digestion stability as a criterion for protein allergencity assessment. Ann. NY Acad. Sci. 964:95-110]. Data according to guidance by FAO/WHO (2001) [FAO/WHO (2001), Evaluation of Allergenicity of Genetically Modified Foods, Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22 – 25 January 2001] with reduced amounts of pepsin should be submitted additionally. • Heat stability data, e.g. for Cry 1F, Cry34Ab1 and Cry35Ab1, are not conclusive because only loss of biological function and not degradation of proteins into non-allergenic | The comments of the MS have been taken into consideration by the Panel when it assessed the allergenicity of the single events and the new proteins expressed in the single events.<br><br>The overall information provided by the applicant based on the weight of evidence approach that was applied allowed the panel to conclude that the allergenicity was unlikely. |

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| | | | breakdown products was assayed. • Bioinformatics analysis was not conducted according to FAO/WHO criteria (window of 6 consecutive amino acids for homology comparisons). Instead other parameters (window of 8 consecutive amino acids for homology comparisons) were employed. This constitutes a less stringent approach. Respective comparisons according to FAO/WHO guidance should also be submitted. More direct tests for allergenicity as recommended in Spök et al. (2005b) [Spök A., Gaugitsch H., Laffer S., Pauli G., Saito H., Sampson H., Sibanda E., Thomas W., van Hage M., Valenta R. (2005), Suggestions for the Assessment of the Allergenic Potential of Genetically Modified Organisms. Int. Arch. Allergy Immunol. 137: 167-180] are therefore considered necessary to be employed. | |
| Austria | Ministry of Health, Family and Youth | D, 07.09 Allergenicity | Assessment of allergenicity of newly expressed proteins For the assessment of allergenic properties of the introduced proteins reference was made to the assessment of individual traits in parental GMO events. Furthermore mostly indirect evidence was used for the assessment. The indicators used were information on the allergenicity of the source material, homology-comparisons of novel proteins to known allergens, digestibility of test proteins in simulated gastric environments, the heat stability of test proteins, and absence of glycosylation of proteins. | General comment on allergenicity risk assessment. |
| Austria | Ministry of Health, Family and Youth | D, 07.10 Nutritional assessment of GM food/feed | GM Maize 1507x59122 is considered to be nutritionally equivalent to non GM-maize based on the comparison of certain constituents and based on results of broiler feeding study with test material from GM Maize 1507x59122. However the control strain used for the feeding trial is identified by a different ID-Code: C-F-03-154C as the control used for comparative analysis (C-F-03-42C). Therefore it is not possible to assess, whether identical control strains have been used. Furthermore no breeding history is supplied to assess whether the strain is | Since maize 1507x59122 is agronomically and compositionally equivalent to the non-GM comparator, the 42-day poultry study is not required by the GMO Panel to conclude on the safety of this maize. The breeding history of the comparator used in the compositional and agronomic studies was provided by the applicant as additional information. |

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| | | | derived from a genetically modified strain, as is apparently the case with the control for another related Stacked Event GM Maize 1507x59122xNK603. According to EFSA guidance non-GMO controls should not be derivatives of genetically modified lines. We therefore request submission of further information on the control strain. Without submission of more specific information by the notifier his conclusion as stated above has to be rejected. | |
| Austria | Ministry of Health, Family and Youth | D, 08 Post-market monitoring of GM food/feed | According to Art. 5 (3) k) of EU-Regulation 1829/2003 a post-market monitoring-plan should be added to the dossier. | The GMO Panel agrees with the applicant on the fact that post-market monitoring of GM food/feed products containing, consisting of or derived from maize 1507x59122 is not necessary. EU-Regulation 1829/2003 asks only for a post-market-monitoring plan, **where appropriate**. |
| Austria | Ministry of Health, Family and Youth | D, 10 Potential changes in the interactions of the GM plant with the biotic… | For environmental risk assessment only unintended release of GM Maize 1507x59122, e.g. accidental spillage, is considered. **It is not clear which specific routes of unintentional release are considered for the conclusions of the notifier**. It is therefore evident whether cultivation of maize seed contaminated with GM Maize 1507x59122 or the effects of transgenic materials still present in faeces of animals fed with GM Maize 1507x59122 products were considered.<br>Regarding conclusions of the notifier concerning effects on human health and animal health which based on insufficient data see comments to "Compositional Analysis" up to "Nutritional Equivalence". The conclusions do not seem to be justified based on data submitted in the dossier. More data should be submitted, specifically: • Tests with appropriate controls including parental GM events in comparison with GM Maize 1507x59122. • Further empirical evidence concerning the observed statistically significant differences for compositional analyses (comparative assessment) and nutritional equivalence of GM Maize 1507x59122. • Direct evidence concerning potential toxicological and allergenic effects of GM Maize 1507x59122. | The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 1507 x 59122 and does not include cultivation.<br><br>Considering the proposed uses of maize 1507 x 59122, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing. Those are the routes of environmental exposure in case of accidental release which were considered by the GMO Panel in its risk assessment. |

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| | | | Otherwise the conclusions by the notifier need to be rejected. | |
| Austria | Ministry of Health, Family and Youth | D, 12 Environmental Monitoring Plan | Case specific monitoring The applicant concludes that based on the submitted risk assessment no identified adverse effects to humans and animals are to be expected. Therefore a case-specific monitoring is not deemed appropriate by the notifier. However, based on the identified shortcomings of the respective assessment this conclusion needs to be better justified. General surveillance The General Surveillance plan is too general in nature. The description of the monitoring plan by the notifier in Chapter 2 ("detailed of the proposed methods for general surveillance") is insufficient and need to be amended. The plan should better specify the surveillance network involved, with regard to the potential risks that are not addressed in full in the risk assessment by the notifier and the measures, which would enable the participating networks to report any specific observations on adverse effects. Specifically for the monitoring of animal health the notifier need to present more details with regard to institutions approached. The monitoring plan therefore has to be considered insufficient. Descriptions of procedures and institutions involved are missing, as well as specific criteria for observatory measures. No information is contained, what is regarded to be an adverse effect, or how effects should be evaluated. No outline is given, how such information is collected and presented, who is collecting this information, and what knowledge and expertise involved persons should have. In conclusion, it is not clear how unanticipated effects in the environment, human and animal health as well as any substantial unintended release of the GMO will be accounted for under the general surveillance plan proposed. In conclusion, the proposed monitoring plan for GM Maize 1507x59122 is insufficient and inadequate for the purpose of general surveillance. The submitted monitoring plan therefore should be | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>See section 6.1.2 of the scientific opinion |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | rejected. | |
| Belgium | Belgian Biosafety Advisory Council | A. General information | Under f) the sentence is found that the genetic modification in 1507x59122 maize does not give rise to any ethical or religious concerns. It is proposed to omit "to any ethical concern" for the following reason: it is not because inserts in 1507x59122 maize do not contain human or animal genes, or because of no differences in composition,food or feed value,absence of toxicity or allergenicity… that there may be no ethical concerns. Ethical concerns may arise from a certain view on nature and human impact on it, based on subjective reasons originating from such a view, and not only based on objective arguments of safety. Even if these arguments giving rise to ethical concerns are completely subjective, the ethical concerns are nevertheless real and have to be taken into consideration in a democracy if they arise in a substantial part of the population. | Outside the remit of the EFSA GMO Panel |
| Belgium | Belgian Biosafety Advisory Council | D, 04 Information on how the GM plant differs from the recipient plant in: … | Table 5 claims in the title to give data on the stacked transgene event and the two parent lines. The table does not give information on the parental lines. | It is confirmed that the heading of Table 5 does not correspond to the data summarised in the table. In its assessment of agronomic data, the GMO Panel referred to the detailed information as set out in Annex 2. |
| Belgium | Belgian Biosafety Advisory Council | D, 05 Genetic stability of the insert and phenotypic stability of the GM plant | Figure 7 in annex 1 is unreadable. On p 11 of Annex 1 the following paragraph is written: "The 1507 inbred line was created by several rounds of backcrossing to the 3KP inbred background and the 59122 inbred line was created by two rounds of backcrossing to the 1W2 inbred background. The stacked hybrid represents a cross of the two inbred lines and contains the 3KPx1W2 hybrid background. Both the 59122 line and the stacked hybrid were expected to segregate for the event DAS-59122-7 insertion." Inbred lines are not expected to segregate; yet it is written that line 59122 is expected to segregate for the event……". How do the applicants explain this ? Although the applicants mention that the pedigree of the hybrid is available, | Pedigree information regarding stacked events is not a requirement according to the Guidance Document on Stacked Events and not relevant for the genetic stability of the insert and phenotypic stability of the GM plant.<br><br>The applicant provided pedigree information as additional information.<br><br>Upon request by the GMO Panel, the applicant provided detailed information on the genetic background of the non-GM control used in the study on agronomic characteristics, quantitative ELISA and nutrient composition analysis of maize 1507x59122 (Annex 2). |

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| | | | no production scheme is given in the dossier. Hence we do not know with which (segregating ????? see comment here above) material trials and tests are conducted. We would like to see a clear history of the pedigree of the final hybrid and of the material used in all trials and tests. | |
| Belgium | Belgian Biosafety Advisory Council | D, 07.04 Agronomic traits | The 1507 x 59122 maize was tested in North America and Canada during 2003. The results obtained confirmed that it is comparable to non-GM control maize, regardless of herbicide treatment. European agronomists never lean on results from trials conducted during 1 single year to compare varieties. Variety trials are always conducted during several years in several locations in order to be able to calculate genotype*environment interactions and to study overyears variability. | Considering the fact, that agronomic equivalence except for the intended traits was demonstrated by the applicant, the GMO Panel considers a field trial over a single growing season as sufficient. |
| Belgium | Belgian Biosafety Advisory Council | D, 07.08 Toxicology | A 42-day feeding study in broiler chickens was performed by using 1507x59122 maize. First, it was checked whether protein content in 1507x59122 maize grains is similar to that in the 1507 and 59122 maize grains respectively. This was indeed the case for the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins. Annex 3 refers to the In this study of Delaney and Smith (2004); 33J56 is a commercially available non-transgenic hybrid maize grain which is used as a reference substance. Table 3 clearly indicates the absence of Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in this 33J56 maize. In table 14, three out of the four proteins are detected (Cry1F, Cry34Ab1, Cry35Ab1; the fact that the PAT protein is not detected is not a surprise, since its concentration is most of the time below the limit of quantitation) in the starter and finisher phase diet of the 33J56 reference group. On p 17-18 of annex 3, it is mentioned that this is due to contamination during clean-up. Any comparison making use of the 33J56 reference group is scientifically incorrect. A 13-week feeding study in the rat is not included. Why not? Such a study should be performed since synergistic effects cannot be excluded beforehand. | Considering the fact that agronomic and compositional equivalence to the non-GM comparator was demonstrated, the GMO Panel does not require a 90-day rat study and a 42-day poultry study to conclude on the safety of maize 1507x59122. |

| Application EFSA-GMO-UK-2005-15 (Maize 1507x59122) ANNEX G<br>Comments and opinions submitted by Member States during the three-month consultation period | | | | |
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| **Country** | **Organisation** | **Reference** | **Comment** | **EFSA GMO Panel response** |
| Belgium | Belgian Biosafety Advisory Council | D, 07.09 Allergenicity | Assessment of the allergenicity of the whole GM plant or crop. In section 7.9.2, the allergenicity of the genetically modified maize has not been investigated. The rationale of this section is not to take the new traits into consideration, but to evaluate, due to the introduction of the new traits, possible changes in the allergenicity of the recipient plant when this plant is known as an allergenic source. Although it is rare, food allergy to maize exists and we must be cautious that it does not become more frequent. Major allergens have been determined (Pastorello et al. 2003; Pasini et al. 2002), and new allergens might be described in the near future (Weichel et al. 2006). Besides the fact that the introduced traits are not likely to behave as allergens, their introduction in the plant and the effects thereof might interfere with the expression levels of other maize proteins, including allergens. For that reason, it is relevant to analyze whether the expression levels of known major allergens is increased in genetically modified 1507x59122 maize grains. This can be carried out with Elisa to purified allergens. It can also be determined whether the overall allergenicity of a genetically modified grain maize extract is increased, as compared to that of its traditional counterpart. Again, Elisa can be used, by using maize patients serum to probe. Pastorello et al. J Allergy Clin Immunol 2003; 112;775-83 Pasini et al. Allergy 2002; 57:98-106 Weichel et al. Allergy 2006;61:128-35 | Single events and newly expressed protein in single events, particularly Cry proteins) have been already assessed, including for allergenicity.<br><br>The Panel is not aware of any new information that would change its opinion.<br><br>In addition the overall information provided by the Applicant does not indicate possible interactions between thse newly expressed proteins that would in particular impact on the allergenicity.<br><br>With regards the allergenicity of the whole plant, the panel is aware of the rare cases of allergy to maize, which however is not considered a common allergenic food. The Panel sees no reason to consider that the allergenicity of the GM maizes (e.g. the single events already assessed as well as the present stack event) would be changed because of the genetic modification |
| Belgium | Belgian Biosafety Advisory Council | D, 12.01 General | We support the recommendation of ACRE (2006) that provision of detailed arrangements for general surveillance post-market monitoring plans for the import and processing of grain from GM maize should be made a condition of any consent. These should include which and when information should be provided to EFSA and how the applicant can ensure this to happen. Although resistance to insect attack is not the only factor preventing maize to grow outside the agricultural environment, the (indeed low) | This comment partially falls outside the remit of the GMO Panel. Decision-making for specific conditions for placing a GMO on the market is left to the risk-managers (e.g. European Commission and Member States).<br><br>The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national |

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| | | | possibility of the establishment of maize protected against insect larvae in the wild in Europe should be a point of particular interest in a more detailed general surveillance plan. ACRE (2006). General advice on notifications for import and marketing of GM maize grain. (http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice74.pdf ) | competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |
| Denmark | Danish Environmental Protection Agency | General comments | Denmark has no comments to the application. | |
| Finland | Board for Gene Technology | General comments | We want to emphasize the need of high quality of general surveillance plan when adopting the product in a specific country. | Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |
| France | MINEFE - DGCCRF | D, 02 Information on the sequences actually inserted or deleted | (D) Informations relatives à la plante génétiquement modifiée (2) Les analyses de type Southern, utilisant une large gamme d'enzymes de restriction et de sondes spécifiques des inserts 59122 et 1507, montrent que les inserts présents chez l'hybride correspondent bien aux inserts hérités de chacun des parents, que la structure moléculaire des inserts tels que décrits chez les parents est préservée chez l'hybride obtenu par croisement conventionnel et que les inserts sont situés dans le génome nucléaire de l'hybride. Cependant qu'aucune information n'est donnée sur le mode de constitution de l'hybride porteur des deux événements de transformation. | The hybrid 1507 x 59122 is constructed by conventional breeding. |
| France | MINEFE - DGCCRF | D, 07.08 Toxicology | 7.8.4) Etude de toxicité subchronique Maïs 1507 x 59122 Aucune étude de toxicité subchronique n'a été réalisée chez le rat avec le maïs hybride 1507 x | Considering the fact that agronomic and compositional equivalence to the non-GM comparator was demonstrated and in particular that there was no |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | 59122 mais que, compte tenu du fait que : - des études de toxicité subchronique de 90 jours ont été réalisées avec les maïs parentaux 59122 et 1507 et qu'aucun effet délétère n'a été observé chez l'animal pour ces maïs, - aucun effet toxique ou délétère chez l'animal de laboratoire n'a été mis en évidence pour les 4 protéines d'intérêt, - les niveaux d'expression des protéines d'intérêt, compte tenu des écart-types observés, n'étant pas modifiés chez l'hybride comparés aux niveaux mesurés chez les parents, un tel élément est en faveur d'une absence d'interaction entre les événements de transformation, - une étude d'alimentarité a été réalisée chez le poulet qui permet de conclure à l'équivalence nutritionnelle du maïs hybride avec son témoin, il est possible de considérer que ces éléments, notamment les résultats des deux essais de toxicité subchronique sur chacun des maïs parents, sont suffisants pour démontrer l'innocuité des produits de l'hybride 1507 x 59122. L'Agence française de sécurité sanitaire des aliments considère qu'au regard notamment des données sur l'analyse des résultats de composition chimique, les données de toxicité chez les parents et de l'étude d'alimentarité chez l'animal cible, les produits dérivés des variétés de maïs portant dans le même génome les événements de transformation 59122 et 1507 présentent le même niveau de sécurité sanitaire que le maïs conventionnel et ses produits dérivés. Il convient cependant de noter qu'aucune information n'est donnée sur le mode de constitution de l'hybride porteur des deux événements de transformation. Cette information, même si elle n'affecte pas l'évaluation des risques de cet organisme génétiquement modifié, devrait être fournie dans le dossier. En effet, dans ce type de dossier où les empilements de gènes sont plus nombreux, une telle information devient nécessaire pour rendre transparente au plan de la génétique formelle les constitutions génétiques et mieux comprendre la pertinence des témoins. | indication of a possible interaction between the different newly expressed proteins, the GMO Panel does not require additional animal studies, e.g. 90-day rat study and/or 42-day poultry study to conclude on the safety/nutritional equivalence of maize 1507x59122. |

| Application EFSA-GMO-UK-2005-15 (Maize 1507x59122) ANNEX G<br>Comments and opinions submitted by Member States during the three-month consultation period | | | | |
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| **Country** | **Organisation** | **Reference** | **Comment** | **EFSA GMO Panel response** |
| Germany | Federal Agency for Nature Conservation (BfN) | General comments | Although application EFSA/GMO/UK/2005/20 does not include the cultivation of 1507x59122 maize in the European Union, possible ecological consequences arising from accidental spillage or other forms of introduction of the transgene products in the environment should be considered thoroughly. In this respect 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). Our comments on EFSA-GMO-NL-2005-15 refer mainly to the new information provided by the applicant with regard to the hybrid. Because the evaluation of this application must rely also on previous evaluations of the parental GM-lines 1507 and 59122 we herewith also refer to the statements of the German Competent Authorities, including comments of the Federal Agency for Nature Conservation, on applications EFSA-GMO-NL-2004-02 and EFSA-GMO-NL-2005-12. | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See sections 6.1.3 of the scientific opinion |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 03 Information on the expression of the insert | (a) Information on the developmental expression of the insert during the lifecycle of the plant Expression of the inserts was analysed by the applicant on the basis of field data from North America (Canada and US sites). Field data rely solely on the 2003 growing season (see Buffington 2004; dossier, Annex II). Although data for different plant tissues were collected (Buffington 2004), only the expression in grain was used for the risk assessment. Expression analysis must be regarded as an important part of the GMO risk assessment because it i) delivers background data to estimate exposure of target and non-target organisms, ii) allows to reflect on the stability of the genetic modification, and ii) indicates possible interactions between the GMO and environmental factors. Moreover, in hybrid GMO (produced via conventional crosses) such as 1507x59122 maize expression data will be important to address interactions between the genetic elements of the parental lines. Because of its prominent role | The applicant provided additional data from European field trials with 1507 x 59122 in 2005, on three locations in Spain. In these trials both parental lines 1507 and 59122 were used as controls. Results include developmental expression of the insert during the lifecycle of the plant. The GMO panel considers the expression data provided and the analyses performed to be sufficient to substantiate expression of the insert.<br><br>As additional information, the applicant provided a study that measured the protein concentrations of Cry34Ab1, Cry35Ab1, Cry1F, and/or PAT in tissues sampled from 1507, 59122, and 1507x59122 maize. The field phase of this study was conducted in Spain and contained three separate field locations. Each location contained maize 1507, 59122 and 1507x59122. Plots of maize 1507, 59122, and 1507x59122 were untreated or treated with two applications of a herbicide containing glufosinate. Throughout the growing season leaf, root, whole plant, |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | expression analysis should be robust including the use of appropriate statistics. We recommend amending the expression analysis: • Descriptive statistics should refer to both mean and range for an initial comparison of GMO and control. Descriptive statistics such as presented in Tables 2-5 (Buffington 2004, pp. 104) should include the actual numbers of samples used. • Descriptive statistics should be followed by tests based on refined statistical models. The latter should be employed to test the influence of herbicide treatment, of location (environment), and of differences between stacked (hybrid) vs. near-isogenic events. The present sample size will need to be increased to allow reliable testing. • Subjective observations such as "comparable", "in the same order of magnitude", "biologically significant" or "consistent with", which were used by the applicant, should be explained or avoided. • Expression data from individual plants as opposed to multiple-plant samples should be used. • The representativeness of the field locations should be explained in detail. For the risk assessment field locations should be chosen in a way to represent all environments where the GMO is grown, covering the full range of climatic conditions. Choosing the main growing regions in this respect can not be considered as adequate. • Since protein expression in plants can be affected by climatic conditions, soil fertility, agricultural practice or unknown gene-environment interactions, data from several growing seasons should be analysed. We suggest to include data from at least three field seasons at the same location (with six locations representing different environmental conditions) to create a more reliable data set. • Expression analysis should not be restricted to grain but include different plant tissues. | pollen, stalk, forage, and grain samples were collected for protein concentration analysis.<br><br>According to the Guidance of stacked events ' *at least one year of field trial data is required, with trials ….in geographical localities representative of the climatic conditions under which such crops will be cultivated'.* The choice for the main growing regions is therefore considered appropriate by the GMO Panel.<br><br>On request of the Panel summary information was supplied on levels of the expressed proteins in forage. The GMO Panel considers the information provided to be sufficient on the basis that the scope of the application covers only food, feed, import and processing |
| Germany | Federal Agency for Nature Conservati | D, 04 Information on how the GM plant differs from the recipient plant in: … | The conclusions with regard to changes in reproduction, dissemination, and survivability of 1507x59122 maize strongly rely on the evaluation of agronomic characteristics (Buffington 2004). | The EFSA GMO Panel is satisfied with study on agronomic characteristics as provided by the applicant. Data on the single events as provided with the corresponding applications were also considered. |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | on (BfN) | | Although the agronomic characteristics addressed by Buffington (2004) do not indicate a potential for differences in reproduction, dissemination, and survivability the selected parameters themselves cannot sufficiently indicate such changes. Data presented on disease incidence and insect damage are of limited value because a range of pesticides were applied. The data set is based on a field design which is – because of the small plot size – not comparable to common agricultural practice. With regard to a final assessment, further information on reproduction, dissemination, and survivability is required. A comparison to the parental lines should be included. The notifier refers to the concept of biological significance without giving the rationale for his assessment. The applicant is therefore asked to provide his concept of biological significance for the parameters in question. | |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 07.08 Toxicology | D.7.8.4. Testing of the whole GM food One of the control groups (33J56) has been contaminated with the respective Cry toxins during the course of the experiment. Contamination levels ranged from 3-8% (see Delaney & Smith pp. 17-18). Contrary to the analysis of the study authors we suggest to exclude the control line 33J56 from the data set. Although Delaney & Smith refer to the statistical analysis (p. 15) no protocol of the statistical analysis is given in the study. The applicant is asked to provide the statistical protocol for the above mentioned study. | See above. With regard to the need of the 42-day poultry study. |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 10 Potential changes in the interactions of the GM plant with the biotic… | Although the applicant acknowledges environmental exposure via accidental loss and spillage, other routes of environmental exposure e.g. via faeces and/or waste material and resulting risks have not been analyzed. The applicant is therefore asked to refer clearly to his analyses given in Annex 2, response 5 of the dossier and to complete the assessment by including of exposure and effects on a case specific basis for 1507x59122 maize. Because of the exposure pathways especially effects on water and soil organisms should be included in the | The scope of the application is for food (e.g. syrup, starch, oil) and feed (e.g. meal, oil) uses, import and processing of maize 1507 x 59122 and does not include cultivation. Therefore, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM maize and with accidental release into the environment of GM seeds during transportation and processing.<br><br>See sections 5.2.1.2 (a) and 5.2.1.4 of the scientific |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | analyses. | opinion |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 12.01 General | Interplay between environmental risk assessment and monitoring The safety of 1507x59122 maize cannot be fully assessed because of deficiencies in the application listed under the comments on chapters D.3 to D.9. More data are needed to achieve a final conclusion in the environmental risk assessment. Depending on the results of an updated environmental risk assessment the conclusions concerning the necessity of a case-specific post-market monitoring may need to be revised. | See section 6.1.2 of the scientific opinion |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 12.01 General | As stated by the applicant, the scope of the application of the 1507x59122 maize is for import, processing and all uses for food and feed. The applicant provides an environmental monitoring plan. This post-market monitoring plan does not fully meet the objectives defined in Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC). Therefore, a plan suitable to meet these objectives is requested. | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 12.02 Case-specific GM plant monitoring | We do not share the opinion of the applicant that a case-specific monitoring is not necessary. As stated by the applicant, during transport, storage, package or processing incidental spillage of 1507x59122 maize can occur. Furthermore, the exposure of the GMO and its Cry proteins to the environment during or after the production process (e.g. through organic waste material or sewage) and during or after animal consumption (e.g. through manure) is possible. Therefore, case-specific monitoring has to focus on pathways, where 1507x59122 maize enters the environment. The applicant is requested to provide a case-specific monitoring plan including detailed | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>See section 6.1.2 of the scientific opinion |

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| | | | information • how losses and spillage of 1507x59122 maize during transport, storage, package, processing and use as feed will be monitored, • how the exposure of organic waste material, sewage or manure containing 1507x59122 maize or Cry proteins to the environment during or after the production process or animal consumption will be monitored. To ensure that the data gained through monitoring are scientifically sound, it is essential, that the applied monitoring scheme includes a statistically sufficient number of samples. In the unlikely case that spread, persistence and accumulation of 1507x59122 maize and the Cry proteins in the receiving environment occur, further observations of possible impacts on organisms, food chains and habitats in the specific environment are required. | |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 12.03 General Surveillance of the impact of the GM plant | The general surveillance plan provided by the applicant is not in line with Annex VII of Directive 2001/18/EC and the supplementing guidance notes (2002/811/EC). The applicant presents a plan that e.g. observations by operators will be used for general surveillance. The professional qualification of these operators is not specified and further details not mentioned. As monitoring parameters the applicant lists: extent of unintended release, environment, management systems, etc. This is too unspecific. The general surveillance plan has to meet the following requirements: A list of monitoring parameters more specific than suggested by the applicant has to be provided. The applicant is requested to present for each parameter a detailed statement of the parameter definition, the observation methods (collection and analysis of samples with references), the frequencies of observations (time and number of visits to collect data) and the monitoring locations including number and size. Furthermore an operating schedule giving full details of points in time is requested. If monitoring data are collected by external people or existing networks the monitoring expertise of the | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |

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| | | | external people involved in the monitoring activities and detailed information about participating networks (e.g. name, EU country, responsible authority, availability, scope of monitoring, composition of the network) have to be specified. Binding agreements/contracts with third parties (external persons and/or existing networks) are requested which clearly determine what data are provided and how these data are made available. The concept of sampling needs to be elaborated. Particularly, it must be explained how the necessary representativeness of the collected data in space and time shall be achieved. The applicant is requested to indicate how the monitoring plan is adapted to different local conditions where appropriate. The methods of data analysis including the statistical methods have to be elaborated in detail. The time-period of monitoring needs to be sufficient to detect delayed or long-term adverse effects. Therefore, it may be necessary to extend the monitoring of certain parameters beyond the period of the consent. Furthermore, the general surveillance plan has to focus on possible pathways how 1507x59122 maize can get into the environment and how unforeseen adverse effects on human health and the environment can be linked to the dispersal of the GMO. | |
| Germany | Federal Agency for Nature Conservation (BfN) | D, 12.06 Reporting the results of monitoring | The applicant is required to report on the results of the monitoring including all issues of case-specific monitoring on an annual basis. All raw data have to be made available if requested. | See section 6.1.2 of the scientific opinion:<br>'*No specific environmental impact of this GM maize was indicated by the environmental risk assessment and thus no case specific monitoring is required.'*<br><br>The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan (on an annual basis). |
| Germany | Federal Office of Consumer Protection and Food | General comments | The scope of application EFSA/GMO/NL/2005/15 covers import and processing of maize 1507 x 59122 including all feed and food products containing, consisting of, or produced from the genetically modified maize 1507 x 59122. Cultivation is not | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | Safety (BVL) | | covered by this application. The Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion that the data provided with the application EFSA/GMO/NL/2005/15 support the conclusion that maize 1507 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. 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. | applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | A, 07 Where appropriate, the conditions for placing on the market the food(s) or… | Products consisting of maize seed should be accompanied by an instruction leaflet including the information that maize 1507 x 59122 has not been approved for cultivation by the EC. Furthermore, it should indicate that resulting plants are able to tolerate herbicides containing glufosinate-ammonium as active ingredient, rendering such herbicides inappropriate for management purposes. Appropriate measures should be taken during transport, storage, and processing to avoid unintended release into the environment. | Human and animal health issues related to plant-protection products are regulated by Directive 91/414/EEC and fall outside the remit of the GMO Panel. The same applies to issues related to labelling. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 02 Information on the sequences actually inserted or deleted | (a) The copy number of all detectable inserts, both complete and partial We wish to point out that Table 15 (mentioned on page 12) can not be found in the technical dossier (part I). Thus, the applicant should be asked to indicate where it can be found in the dossier or to provide this table. | Data provided by the applicant demonstrate that the stacked line contains one copy of each event. No additional partial copies were found.<br><br>On page 12 in the technical dossier a summary of data on the single event 1507 is given. For the original data on 1507 the applicant refers to other earlier supplied data and opinions of the GMO Panel on 1507 (EFSA, 2004 and 2005a, b). |
| Germany | Federal Office of Consumer Protection and Food Safety | D, 03 Information on the expression of the insert | (a) Information on the developmental expression of the insert during the lifecycle of the plant Data on the expression of the inserts of the stacked events were obtained from field trials at five locations in the US and Canada in 2003. No appropriate comparator (parental lines) was grown in this field trial. Instead, | The applicant provided additional data from European field trials with 1507 x 59122 in 2005, on three locations in Spain. In these trials both parental lines 1507 and 59122 were used as a control. Results include developmental expression of the insert during the lifecycle of the plant. The GMO panel considers the |

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| | (BVL) | | obtained data for the stacked events were compared with data obtained from parental lines grown in field trials in the US, Canada and Chile at different locations and in different growing seasons which is not in line with the EFSA Guidance document on stacked events (EFSA, 2007). Furthermore, the dossier discusses the expression analysis of the transgenes in grain only. Data on forage and other plant tissues are available (Buffington, 2004), but were not compared to appropriate comparators and discussed. Therefore, the applicant should be requested to deliver comparative information regarding the protein levels in other relevant parts of the plant (at least: forage). Buffington, J. (2004) Agronomic Characteristics, Quantitative ELISA, and Nutrient Composition Analysis of Hybrid Maize Lines Containing cry1F, cry34Ab1, cry35Ab1, and pat Genes: U.S. and Canada Locations. Study number PHI-2003-017. Unpublished technical report. Pioneer Hi-Bred International Inc. EFSA (2007) Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assess¬ment of genetically modified plants containing stacked transformation events. The EFSA Journal 512, 1-5. | expression data provided and the analyses performed to be sufficient for the safety assessment.<br><br>Study on expression levels: see above for the complementary data provided as additional information.<br><br>On request of the Panel summary information was supplied on levels of the expressed proteins in forage. The GMO Panel considers the information provided to be sufficient on the basis that the scope of the application covers only food, feed, import and processing. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 04 Information on how the GM plant differs from the recipient plant in: … | The title of Table 5 does not comply with the data shown in Table 5. Contrary to the headline information, only mean agronomic data from 1507 x 59122 maize sprayed with glufosinate-ammonium and from unsprayed non-GM control are presented. In contrast, data from both parental lines as well as from unsprayed GM maize in general are missing. Thus, the applicant should be requested to complete the table by adding data from both parental lines (sprayed with glufosinate-ammonium and unsprayed) just as from unsprayed 1507 x 59122 maize. | See above |
| Germany | Federal Office of Consumer Protection and Food | D, 05 Genetic stability of the insert and phenotypic stability of the GM plant | Contrary to the applicants' statement, the results summarized in Table 5 are not appropriate to confirm the stability of both inserts in 1507 x 59122. The presented data only demonstrate that the GM plant does not differ from the recipient plant in common | The remark of Germany is correct. However, other data on genetic stability of the insert are considered sufficient to conclude on the stability of the events in the stacked line. |

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| | Safety (BVL) | | phenotypic and agronomic characteristics but do not extend to qualities connected with the expression of the inserts. A comment by the applicant on the statement quoted above should be requested. | |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 07.04 Agronomic traits | In order to demonstrate that the agronomic characteristics of maize 1507 x 59122 are comparable to non-GM control maize, regardless of herbicide treatment, the applicant refers to the mean agronomic data shown in Table 5. We wish to point out that Table 5 is not appropriate to confirm this statement as data from unsprayed GM maize are missing. The applicant should be asked to complete Table 5 by adding the missing agronomic data from unsprayed GM maize. | Extensive agronomic data on unsprayed 1507x59122 maize can be found in Annex 2. |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 07.08 Toxicology | D.7.8.1. Safety assessment of newly expressed proteins In order to demonstrate the safety of the proteins Cry34Ab1 and Cry35Ab1 a mixture of both proteins was evaluated for acute oral toxicity from gavage administration to five male and five female CD-1 mice (Brooks and DeWildt, 2000). In this context, we wish to point out that the attached study report (Brooks, 2000) is not consistent with the cited reference as it refers not to Cry34/35Ab1 but to the PAT protein. Therefore, the applicant should be asked to provide the appropriate study report. Brooks, K.J. and DeWildt, P.M. (2000) PS149B1 14KDa protein: Acute oral toxicity study in CD1 mice. Study number 001128. Unpublished technical report. Dow Chemical company. Brooks, K.J. (2000) PAT microbial protein (FL): Acute toxicity study in CD-1 mice. Study number 991249. Unpublished technical report. Dow Agrosciences LLC. D.7.8.4. Testing of the whole GM food The applicant describes a 42-day feeding study using broiler chickens. The report of this study (Delaney and Smith, 2004) is missing relevant details. The study report presented merely incomplete raw data. Only analysis of whole groups but no individual data are shown. Furthermore, a sex-specific analysis is missing. Moreover, both data on mortality and on carcass yields are not presented | The safety of the newly expressed proteins (particularly Cry proteins) was assessed by the GMO Panel within the applications for the single events.<br><br>The Panel is not aware of any new information that would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between those newly expressed proteins that would in particular impact on the food/feed safety.<br>Since maize 1507x59122 is agronomically and compositionally equivalent to its non-GM control, no toxicological studies with laboratory animals nor nutritional studies, e.g. 90-day rat study and/or 42-day poultry study, are required to conclude on the safety/nutritional equivalence of maize 1507x59122. |

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| | | | in absolute numbers. Besides, Appendix 2 (page 51-214) is missing completely. The available data presented by the applicant do not indicate the occurrence of adverse effects. However, a final assessment of the results is not possible. Therefore, the applicant should be requested to deliver an entire version of the study report of the poultry feeding study including all raw data (particularly with regard to individual data on body weights and on mortality) as well as a sex-specific analysis of data. Minor Comment: 42-day poultry feeding study: Some of the presented PCR analysis which were performed to confirm the identity of maize 1507 x 59122 and the absence of the cry1F, cry34Ab1, cry35Ab1, and pat genes in the control and reference substances are of minor quality. Besides weakly positive results in some of the control and reference samples, some of the test samples result in hardly visible signals that do not allow for a clear classification of the investigated material. Delaney, B.F. and Smith, B. (2004) Nutritional Equivalency Study of Stacked Hybrid of Transgenic Maize Line 1507 (Event DAS-Ø15Ø7-1) and 59122 (Event DAS-59122-7): Poultry Feeding Study. Internal unpublished report of Pioneer Hi-Bred International study ID: PHI-2003-047. | |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 07.09 Allergenicity | D.7.9.1. Assessment of allergenicity of the newly expressed protein In order to demonstrate that the Cry1F protein is rapidly degraded under simulated gastric fluid digestive conditions the applicant refers to the studies of Korjagin and Ernest (2000) and Schafer and Korjagin (2001). In this regard, we wish to point out that the study of Korjagin and Ernest (2000) is inappropriate to prove that statement as the test was conducted under simulated intestinal fluid digestive conditions. Furthermore, the study came to the conclusion that the Cry1F protein remained undigested in simulated intestinal fluid for the duration of the assay (120 min). Korjagin, V.A. and Ernest, A.D. (2000) In vitro simulated intestinal fluid digestibility study of microbially-derived Cry1F. | The comments of the MS have been taken into consideration by the Panel when it assessed the allergenicity of the single events and the new proteins expressed in the single events (particularly Cry proteins).<br>The overall information provided by the applicant based on the weight of evidence approach that was applied allowed the panel to conclude that the allergenicity was unlikely.<br>The Panel is not aware of any new information that would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between these newly expressed proteins that would in particular impact on the allergenicity. |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | Study number GH-C 5146. Unpublished technical report. Dow AgroSciences LLC. Schafer, B.W. and Korjagin, V.A. (2001) In vitro simulated gastric fluid digestibility study of truncated Cry1F delta-endotoxin derived from Pseudomonas fluorescens. Study numberGH-C 5367. Unpublished technical report. Dow AgroSciences LLC. In order to prove that the protein Cry1F is heat labile, the applicant cites a study by Herman (2000). In this context, we wish to point out that application EFSA/GMO/NL/2005/15 comprehends two studies by Herman (2000), both of which refer to tests conducted with Cry34Ab1 and Cry35Ab1 but not Cry1F. Therefore, the applicant should be asked to provide evidence which corresponds to the heat lability of Cry1F. Herman R. (2000) Thermolability of PS149B1 binary delta-endotoxin. Study number 001041. Dow AgroSciences. Herman, R.A. (2000a) Microbial PS149B1 binary delta-endotoxin: maize-insect-pest susceptibility study. Study number GH-C 5114. Unpublished technical report. Dow AgroSciences LLC. | |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 10.04 Interactions between the GM plant and target organisms | With regard to Table 9, we want to indicate that an explanatory legend is missing. Thus, the applicant should be asked to define the indices used in Table 9 (*, a, c). | (-) |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 12.03 General Surveillance of the impact of the GM plant | The general surveillance plan is more or less acceptable, but needs some modifications. As part of the “active surveillance”, it is planned to inform traders and processors as well as to gather information from different communication networks. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried and gathered. The use of questionnaires could be an appropriate measure to survey this information. In addition, it might be useful to integrate food and feed surveillance in coordination | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information |

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| | | | with the competent authorities. Information about the use of the product in food and feed could deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Furthermore, the applicant should specify monitoring activities in the field of human and animal health. Therefore, it should be described in more detail how animal and human health surveillance is integrated in the monitoring plan. A report on GS activities only every third year is not sufficient. The applicant should report on an annual basis about the conducted monitoring measures and every third year an extended report with an overall analysis of the results form the last years. However, the monitoring reports should not only consist of general information from participating networks, but should also be analysed by the consent holder in more detail. In particular, indirect, long-term or cumulative effects could be detected after consideration of data from different networks and overall analysis over several years. Possibly single participating networks will not be able to take this aspect into consideration. | provided.<br><br>See section 6.1.2 of the scientific opinion |
| Germany | Federal Office of Consumer Protection and Food Safety (BVL) | D, 12.06 Reporting the results of monitoring | A report on GS activities only every third year is not sufficient. The applicant should report on an annual basis about the conducted monitoring measures and every third year an extended report with an overall analysis of the results form the last years. | See section 6.1.2 of the scientific opinion<br><br>The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan (on an annual basis). |
| Greece | Hellenic Food Authority (E.F.E.T.) | D, 02 Information on the sequences actually inserted or deleted | Since the probes used in southern blots confirming the copy number, the structure and the organization of the inserts in 1507 X 59122 are not adequately designed (they do not include flanking regions as proposed by EFSA guidance), additional molecular analysis data should be provided in order to further complete the molecular characterization. PCR analysis, southern blots using properly designed probes or sequence analysis of the insert and its flanking regions should be performed in order to confirm the organization of the insert into the hybrid | Additional information has been requested on the intactness of the inserts and the flanks.<br>Molecular equivalence of the 1507 and 59122 inserts in the hybrid line was determined by Southern analysis, using *Sac*I and *Hind*III digested genomic DNA and probes of the *pat*, *cry*34Ab1, *cry*35Ab1 and *cry*1F genes. From the hybridisation patters of 59122 x 1507 and both parental lines it was concluded that the organisation of sequences in the insert are unchanged. Also the intactness of the 1507 insert and of the 3' side of the 59122 was confirmed. |

| Application EFSA-GMO-UK-2005-15 (Maize 1507x59122) ANNEX G<br>Comments and opinions submitted by Member States during the three-month consultation period | | | | |
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| **Country** | **Organisation** | **Reference** | **Comment** | **EFSA GMO Panel response** |
| | | | genome. | Additional information has been supplied on the intactness of the 5' of the 59122 insert in the hybrid line. The intactness of the 59122 insert in the hybrid line was confirmed by results obtained by event-specific real time PCR of the 5' region of the 59122 insert. |
| Greece | Hellenic Food Authority (E.F.E.T.) | D, 07.02 Field trials \| D, 07.04 Agronomic traits \| D, 07.10 Nutritional assessment of GM food/feed | For the comparative assessment (agronomic, compositional and nutritional studies) as comparators were used only the non-GM nearly isogenic lines and commercial hybrids (Annex 2 and Annex 3) but not the GM parental lines as it is clearly indicated in EFSA's Guidance Document for stacked genes (The EFSA Journal, 2007, 512:1-5). There should be provided to EFSA the comparative assessment with the use of the GM parental lines as comparators. | See above. As regards comparative agronomic and compositional data, the GMO Panel considered the data provided within the applications for the single events. |
| Greece | Hellenic Food Authority (E.F.E.T.) | D, 07.08 Toxicology | Each of the introduced traits from the parental lines are inherited in 1507 × 59122 maize, which results in the expression of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins in the same plant. There should be conducted a toxicity study (a 90-day rat feeding study) with the whole of the 4 introduced proteins in the hybrid and not with each one transformation event (GM parents) separately. | As agronomic and compositional equivalence between maize 1507x59122 and its non-GM comparator was demonstrated, the GMO Panel does not require a 90-day feeding study to conclude on the safety of the stacked event. |
| Norway | Directorate for nature management | D, 09 Mechanism of interaction between the GM plant and target organisms (if… | The notifier states "… proteins conferring resistance to certain lepidopteran and coleopteran insect pests cannot be considered as a selective advantage outside the agricultural environment". In our view the Notifier underestimates the change in allele-frequency in populations even small selective advantages may cause over generations. | See section 6.1.2.4 of the scientific opinion "*The GMO Panel considers that the level of exposure of any potential non-target organisms to the CRY proteins expressed in maize 1507 x 59122 in combination with the PAT protein is likely to be very low and of no ecological relevance*". |
| Norway | Directorate for nature management | D, 12.03 General Surveillance of the impact of the GM plant | After reviewing the presented monitoring plan we would like to have seen a higher level of comprehensiveness, detail and specificity on several parts of the monitoring plan. Of special interest would more detailed descriptions of which operators the Notifier intends to use, whether the Notifier will pro-actively gather information from the operators, the nature of the guidance and reporting procedures, and which monitoring parameters the surveillance plan would include in case of substantial loss or spillage of | The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. |

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| | | | the hybrid | The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |
| Norway | Directorate for nature management | D, 12.03 General Surveillance of the impact of the GM plant | The Notifier has proposed a time-period for surveillance of ten years, in line with the authorisation period for the hybrid. Council Decision 2002/811/EC states under 1. Monitoring strategy; "Delayed effects refer to effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effect either at a later stage or after termination of the release. The build-up of resistance by insects to the Bt-toxin through continued exposure is an example of a delayed effect". Further under 1.5. Time-period; "It should also be considered whether it is necessary to extend the monitoring plan beyond the period of the consent". We would like to know what considerations the Notifier has done in this respect; of special interest is the reasoning why an extension of the monitoring plan was found unnecessary. We find the time-limitation in the monitoring plan undesirable, as negative effects can persist even if the product is removed from the market. The time-period of monitoring needs to be sufficient to detect delayed or long-term adverse effects. | |
| Norway | Directorate for nature management | D, 07.09 Allergenicity | 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 1507x59122 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 Cry34Ab1, | The comments of the MS have been taken into consideration by the Panel when it assessed the allergenicity of the single events and the new proteins expressed in the single events (particularly Cry proteins).<br><br>The overall information provided by the applicant based on the weight of evidence approach that was applied allowed the panel to conclude that the allergenicity was unlikely.<br><br>The Panel is not aware of any new information that |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | Cry35Ab1 and Cry1F proteins in food and feed from maize 1507x59122, cannot be excluded. Thus, the Panel's view is that as the adjuvant effect of Cry34Ab1, Cry35Ab1 and Cry1F with reasonable certainty cannot be excluded, the applicant in relation to a possible adjuvant effect of Cry34Ab1, Cry35Ab1 and Cry1F must comment upon the mice studies showing humoral antibody response of Cry1A proteins. Further, although the Cry34Ab1, Cry35Ab1 and Cry1F 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. References: Moreno-Fierros L, Ruiz-Medina EJ, Esquivel R, López-Revilla R, Piña-Cruz S., 2003. Intranasal Cry1Ac protoxin is an effective mucosal and systemic carrier and adjuvant of Streptococcus pneumoniae polysaccharides in mice. Scand J Immunol., 57: 45-55. Prasad S.S.S.V. & Shethna, Y.I., 1975. Enhancement of immune response by the proteinaceous crystal of Bacillus thuringiensis var thuringiensis. Biochem Biophys Res Commun., 62: 517-521. Rojas-Hernández S, Rodríguez-Monroy MA, López-Revilla R, Reséndiz-Albor AA, Moreno-Fierros L., 2004. Intranasal coadministration of the Cry1Ac protoxin with amoebal lysates increases protection against Naegleria fowleri meningoencephalitis. Infect Immun., 72:4368-4375 Vazquez-Padron RI. Martinez-Gil AF. Ayra-Pardo C. Gonzalez-Cabrera J. Prieto-Samsonov DL. de la Riva GA., 1998. Biochemical characterization of the third domain from Bacillus thuringiensis Cry1A toxins. Biochem Mol Biol Int., 45(5):1011-20. Vazquez RI. Moreno-Fierros L. Neri-Bazan L. De La Riva GA. Lopez-Revilla R., 1999. Bacillus thuringensis Cry1Ac protoxin is a potent systemic and mucosal adjuvant. 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 | would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would in particular impact on the allergenicity. |

| Country | Organisation | Reference | Comment | EFSA GMO Panel response |
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| | | | proteins in the mouse small intestine. Biochem Biophys Res Commun., 271:54-8. Vazquez-Padron RI. Moreno-Fierros L. Neri-Bazan L. Martinez-Gil AF. de-la-Riva GA. Lopez-Revilla R., 2000b. Characterization of the mucosal and systemic immune response induced by Cry1Ac protein from Bacillus thuringiensis HD 73 in mice. Braz J Med Biol Res., 33: 147-55. | |
| Spain | NATIONAL COMMISION ON BIOSAFETY | A. General information \| D, 02 Information on the sequences actually inserted or deleted \| D, 07 Information on any toxic, allergenic or other harmful effects on human or... \| D, 07.09 Allergenicity \| D, 12.01 General \| D, 12.03 General Surveillance of the impact of the GM plant | SPANISH COMMENTS EFSA/GMO/NL/2005/15 : 1507 x 59122 MAIZE Comments of the National Commission on Biosafety of Spain General comments Due to the fact that a specific analytical method for the detection and quantification of this hybrid has not been provided yet, Spain is concerned about the legal and administrative implications which could come out from the analytical results in the final product in order to identify and quantify the parental lines (1507 and 59122) and the hybrid (1507 x 59122) separately. Concerning to Certified Reference Material (CRM), the applicant enclose this information for each event only considering that both of these methods can be applied for detection of their particular trait in the hybrid. Although at the moment this analytical methodology is accepted, we consider that the availability of the Reference Material for the hybrid is needed to evaluate the certainty of the individual methods for the hybrid. D.02. Information on the sequences actually inserted or deleted The molecular characterisation is complete and the results can be considered satisfactory, although we suggest that it could be desirable to have Southern analysis of better quality. D.7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food and feed D.07.09 - Toxicology. The notifier should submit sub-chronic studies with both proteins together since we consider that studies made with proteins separately are not enough for carrying out the overall risk assessment of these GMO products. We believe that the current EFSA Guidelines document for the hybrids is not | First part of the comment: outside the remit of the GMO Panel<br><br>Single events and newly expressed protein in single events, particularly Cry proteins) have been already assessed for food/feed safety, including allergenicity. The Panel is not aware of any new information that would change its opinion.<br>In addition the overall information provided by the Applicant does not indicate possible interactions between the newly expressed proteins that would impact on the food/feed safety and allergenicity.<br><br>The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion<br><br>D.12 and D.12.03 The GMO Panel comments on the scientific quality of the monitoring plan. EFSA has published guidance and opinion on PMEM (EFSA, 2006a,b) following a broad consultation with |

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| | | | enough detailed in this sense. D.12. Monitoring Plan D.12.03 - General Surveillance of the impact of the GM plant. The consent holder should provide further details of the arrangements of the monitoring plan, in particular for general surveillance, indicating which existing network programs could be used, the type of information that should be collected and a more detailed monitoring methodology in order to have a monitoring plan which could be implemented in a harmonised manner among the importer Member States. | stakeholders, including national competent authorities. The information supplied by the applicant is in line with the guidance.<br><br>Upon request of the GMO Panel, the applicant provided further clarifications as regards practical and detailed arrangements for the general surveillance activities. The GMO Panel was satisfied with the information provided.<br><br>See section 6.1.2 of the scientific opinion |