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# SCIENTIFIC REPORT OF EFSA

## Public Consultation on the Updated Guidance Document of the Scientific Panel on Genetically Modified Organisms (GMO) for the risk assessment of genetically modified plants and derived food and feed [1]

## Prepared by the GMO Unit

## (Question No EFSA-Q-2003-005E)

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# OUTCOME OF THE PUBLIC CONSULTATION ON THE UPDATED GUIDANCE DOCUMENT OF THE SCIENTIFIC PANEL ON GENETICALLY MODIFIED ORGANISMS (GMO) FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

## Background

On 22 May 2008, the EFSA Scientific Panel on Genetically Modified Organisms (GMO) adopted the draft updated version of its Guidance Document for the risk assessment of genetically modified plants and derived food and feed (*Annex A: Updated Guidance Document for the Risk Assessment of Genetically Modified Plants and derived food and feed*), in the light of several years of experience in the risk assessment of GMO applications and of the outcome of the Panels' self-tasking activities. In line with EFSA's policy on openness and transparency, and in order to allow the scientific community and stakeholders to comment on its work, EFSA consulted the public on this document. On 21 July 2008 the draft updated guidance document was published for public consultation until the 21 of September 2008.

The updated guidance document formed the basis for the establishment of a legal framework for EFSA's GMO assessment by the European Commission (EC) and the Member States. In the frame of enhancing that process, the comments received during this consultation were forwarded to the EC. EFSA committed to publish an evaluation report on the comments received.

A draft working document for the establishment of this legal framework was prepared by the EC based on the updated guidance of the GMO Panel, on comments of stakeholders and Member States which were provided during the public consultation of EFSA as well as further discussions. EFSA was formally consulted on this document by the Commission on 23 February 2009 and adopted the document with proposed modifications (*see Annex B: Answer from EFSA to the consultation of DG SANCO on Rules for applicants for the preparation and presentation of applications to be submitted under Regulation (EC) No 1829/2003 on GM food and feed*) [2].

[2] EFSA register of questions: Question number EFSA-Q-2009-00500

## Comments received

At the deadline of its public consultation EFSA had received 357 submissions, from 19 interested parties (non-governmental organisations, industry organisations and national assessment bodies and competent authorities). Only comments provided in the electronic template were taken into account. The list of the comments received can be found – without reference to individual submitters – as Annex C of this report.

## Screening and evaluation of comments received

### General

In order to facilitate the evaluation and assessment, all comments were grouped into sections. The section with comments related to molecular characterisation was discussed by the standing molecular characterisation Working Group of the EFSA GMO Panel while the section of Food/Feed safety assessment was discussed by the standing Working Group on Food and Feed safety of the EFSA GMO Panel. The latter Working Group dealt also with the comments on general parts of the document. The majority of comments on the statistical approach and the field trial design have been addressed by the Working Group on statistical considerations in the risk assessment of GM plants of the EFSA GMO Panel.

This report provides a summary of the main issues of concern and their consideration.

### Types of comments

Several comments were related to political and management issues. These comments were considered to be outside the scientific remit of EFSA and were not addressed.

Although the current update of the Guidance Document focused on Food and Feed aspects, multiple comments relating to the sections of environmental risk assessment were also received. Additional consultation on these sections is foreseen after their update on issues such as assessing potential long-term environmental effects of GMO cultivation, in the frame of a mandate from the European Commission[3], and potential risks to non-target organisms by traits such as insect-resistance in GM plants, in the frame of a current self tasking activity.

Comments on allergenicity are not addressed in this report. The EFSA GMO Panel is currently working on a self task activity entitled "the assessment of allergenicity of GM foods/feed" where valid comments that are not addressed in Annex B, will be considered. The document produced by the self tasking Group will be available for public consultation during the course of 2009.

In some comments it was suggested to use methodologies and technologies which are still under development for the risk assessment of GM plants. The Panel critically reviews the published literature and may suggest such studies and accept additional data obtained from ‘non routine’ studies on a case by case basis.

[3] EFSA register of questions: Question number EFSA-Q-2008-262

A large number of comments on the statistical approach and the field trial design have been received as this section was extensively revised. Therefore, the provided responses to these comments are more detailed.

Many comments received were very appropriate and of value for the GMO Panel. These comments have been incorporated in Annex B and they enhance the scientific quality and clarity while providing better understanding of the document.

**Summarised responses to comments related to general sections**

This section responds, in a summarised form, to the main issues raised on the general sections of the Annex A. Annex B takes into account most of these issues in particular in its Part I and Part II, sections 2 and 3.

A number of comments were related to the definitions. The Panel agreed with the deletion of the definitions on hazard, risk, risk analysis, risk assessment, risk management and risk communication, provided as footnote 4 in the Annex A, as these are clearly defined in the General Food Law. Definitions on hazard identification, hazard characterisation, exposure assessment and risk characterisation were based on the definitions provided in the Report of the Scientific Steering Committee's Working Group on Harmonisation of Risk Assessment Procedures in the Scientific Committees advising the European Commission in the area of human and environmental health (SSC 2000). The word integrative in the title of chapter IV is used to highlight the integrative nature of risk characterisation and it does not aim to create a new term. However, the definitions provided by the *Codex Alimentarius* Commission included in Annex B are of equal standards to those provided by SSC and acceptable to the Panel.

In a number of comments the comparative approach and the risk assessment of GMOs outlined by the GMO Panel in Annex A, were questioned. The GMO Panel wishes to clarify that the required testing depends not only on the outcome of the comparative analysis, but also on the outcome of the molecular characterisation, and the available knowledge with respect to the source, function/activity/toxicity and history of human/animal consumption of newly expressed proteins (see Annex B, part II section 1.4.1.). In addition, it depends on the information available on other new constituents and/or natural constituents of which the levels have been altered, in particular on the physiological function and/or toxic properties of these constituents (see Annex B section 1.4.2 and 1.4.3.). Annex A is based on internationally agreed principles and available methodologies. In general EFSA agrees that there is a need for continuous follow up in order to further improve the assessment approach. The EFSA GMO Panel notes that the hazard identification and characterisation and the exposure assessment are the two pillars of the risk assessment process, and the information requirements are interrelated. Annex B is revised to provide better understanding of the approach.

In a few comments it was suggested to tailor the risk assessment into decision trees. However, the Panel considers that the risk assessment should be carried out on a case-by-case basis, while decision trees may not cover adequately specific cases.

**Summarised response to comments related to molecular characterisation**

This section responds in a summarised form to the main issues raised in the field of molecular characterisation in Annex A. The updated text can be found in Part I, Section 3.1 and in Part II, Sections 1.1 – 1.2 of Annex B.

*General risk assessment issues and recommendations*: Several comments were targeted to the safety assessment of antibiotic resistance marker (ARM) genes possibly retained in genetically modified plants. The section briefly discussing this issue is now found in Part I, Section 3.1 of Annex B. EFSA has also released in-depth reviews of this topic which can be downloaded from the EFSA website[4].

*Stacked events*: Comments regarding stacking of single events focused mainly on possible interactions between newly expressed proteins and the levels of proteins in the stacked event compared to the single events. The principal concepts of molecular characterisation of stacked events are outlined in Part I, Section 3.2 of Annex B. Comparison of protein expression data from the single events and the stacked event shall be provided for the risk assessment (see Annex B, Part II, Section 1.2.2.3.h). Interaction between the newly expressed proteins should be also viewed in a broader context as single events often express multiple proteins (e.g. herbicide tolerance + insect resistance). Owing to the high importance of this issue, the assessment of potential interactions between newly expressed proteins is foreseen at several places of Annex B, such as in Part II, Sections 1.4.1, 1.4.4.1.c and in Section 3.2.3.

*Information relating to the genetic modification*: Public comments on this chapter were mainly related to the description of helper plasmid (if used during the transformation process), to the use of carrier DNA during transformation and to the assessment of deliberate alteration(s) in the corresponding sequences in the donor organism(s). The requirement for the description of the helper plasmid (if used) has been reintroduced to Annex B (see Part II, Section 1.2.1.1.d). If carrier DNA were to be used during the transformation, Annex B, Part II, Sections 1.2.1 and 1.2.2.2.a would be applicable regarding the characterisation of DNA actually inserted in the plant. Assessment of deliberate alterations(s) in the corresponding sequences in the donor organism(s) is carried out at different levels. Molecular characterisation data of the GM plant should identify these deliberate and/or unintended change(s) (see Annex B, Part II, Sections 1.2.1.3.a and 1.2.2.2.b). In addition, sequence similarity searches should be performed in order to identify any possible relationship of these gene products with known toxins, antinutrients and allergens (see Annex B, Part II, Section 1.2.1.3.c). These gene products are also subject to food and feed safety assessment, which is described in detail in Annex B, Part II, Sections 1.4 - 1.6.

*Information on the sequences actually inserted/deleted*: Some comments suggested that complementary methods (e.g. quantitative PCR) should also be used for the copy number determination of the transgenic insert(s). While not debating the usefulness and sensitivity of

[4] A) Statement on the safe use of the nptII antibiotic resistance marker gene in genetically modified plants by the Scientific Panel on genetically modified organisms (GMO), http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620775641.htm B) EFSA (2004) Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants (Question N° EFSA-Q-2003-109). *The EFSA Journal, 48, 1-18.*

these new techniques, the GMO Panel considers that a properly designed Southern analysis with proper controls should be the primary method for the detection of all complete and/or partial, possibly rearranged or tandem-repeated inserts. Other comments proposed the routine requirement of the determination of chromosomal location(s) of the insert(s). This information is not considered necessary for the risk assessment in all cases, however, the subcellular location of the insert should always be provided (see Annex B, Part II, Section 1.2.2.2.c). Several comments referred to the characterisation of the (pre)insertion locus(i) and to the related bioinformatics analyses. These analyses serve two purposes: (i) the identification of possible interruption of any known ORFs or regulatory regions by the genetic modification; (ii) the identification of possible creation of any new ORFs as a result of the genetic modification possibly raising safety issues. Annex B introduces a number of new elements and clarifications in this section. Sequence information should be provided for both flanking regions of the insert(s) to allow for the analysis detailed in (i). Bioinformatics databases should be up-to-date with the identification of their characteristics and versions (see Annex B, Part II, Sections 1.2.2.2.e and f). The sequence similarity search for (i) should also use databases containing sequences from other species than the transformed plant. As for (ii), the definition of ORFs is now standardised; all sequences between stop codons, not limiting the length of the sequence should be considered as an ORF. In addition, bioinformatics searches should be conducted on the possible new ORFs not just at the insert-genomic DNA junctions, but also at the junction sites arising due to internal rearrangements of the insert(s) (see Annex B, Part II, section 1.2.2.2.f). Expression analysis of potential new ORFs identified at the junction sites created as a result of the genetic modification shall be provided only in cases when complementary information (e.g. potential for transcription/translation and similarity to known allergens/toxins) indicates a potential safety issue (see Annex B, Part II, Section 1.2.2.3.d).

*Information on the expression of the insert*: Public comments highlighted three main issues: (i) data requirement on protein expression levels, (ii) number of seasons/generations to be presented in relation to trait stability and (iii) conditional requirement of data on RNA level(s). Protein expression data should be derived from field trials and be related to the conditions in which the crop is grown. The range of concentrations of newly produced proteins or existing plant proteins deliberately modified should also be provided. As a new element, expression analysis could be carried out in parallel with compositional analysis (see Annex B, Part II, Sections 1.2.2.3.e and f). Regarding data on trait stability, both genetic and phenotypic stability of the introduced trait(s) should be demonstrated. The request for data from five generations/vegetative cycles has been changed i.e. to indicate the need of data from multiple generations (see Annex B, Part II, Section 1.2.2.4). Finally, RNA expression levels are continued to be asked only on a case-by-case basis, taking into account the characteristics of the insert (e.g. inserts targeted to gene silencing).

**Summarised responses to comments related to field trial design and statistical analysis**

This section responds in a summarised form to the main issues raised on the field trial design and statistical approach proposed in Annex A. A revision of the proposed statistical approach, which takes into account relevant comments received during the public consultation, can be found in section 1.3.2 of the Annex B. A more detailed discussion of the whole issue can also be found in the report of the statistics working group of the GMO Panel, which will be published on the EFSA website by the end of May 2009.

In general the proposed approach was well accepted. There was clearly a need for clarifications to the methodology and calculations required for dossiers.

The main focus of the new proposals for both field trial design and statistical analysis was centered around the following issue: how potential differences in composition between GM plants and their conventional counterparts could be placed into the context of the natural variation due to biological and environmental factors, such as the variation due to the genetic backgrounds of commercial varieties with a history of safe use, whilst allowing for the usual uncertainties associated with the limited availability of data.

Many comments received during the public consultation requested clarification as to whether the proposed statistical approach related to animal feeding studies as well as to plant compositional data. The proposed approach does not relate to the experimental design of animal feeding trials with whole GMO foods/feed, for which EFSA has issued recent guidance separately. However, it might be used for the statistical analysis of data from such animal feeding trials with whole GMO foods/feed, particularly if commercial varieties have been included in those trials, where appropriate and on a case-by-case basis.

Some comments requested clarification whether agronomic/phenotypic data was included within the scope of the new proposed approach. The approach is designed to address those agronomic/phenotypic endpoints that can be measured and included within the same field trials as for compositional analysis.

In response to several requests for further clarification concerning what conclusions should be drawn in the event of endpoints showing difference or non-equivalence, note that:

(i) Neither difference nor non-equivalence by themselves necessarily imply lack of safety;
(ii) When any difference or lack of equivalence is found this should be evaluated in the context of a risk assessment process and interpreted within a risk assessment framework;
(iii) The function of the proposed statistical methodology is to produce results that may be interpreted by biologists, toxicologists or other safety experts and not, at the current state of development, to provide a decision-theoretic framework that allows direct inferences on safety;
(iv) The GMO Panel and its Statistics WG are aware that setting the size of the difference test at the 10% level will lead to a large proportion of tests being found to be significant by chance alone. *Per se* a large proportion of significant differences is not considered a sufficient reason for safety concern, unless the proportion would be larger than the proportion of significant results which can be expected for differences between two randomly chosen commercial varieties. Safety concerns may also be raised if the differences follow some systematic pattern such that endpoints of a certain type form a cluster that are significant;
(v) Annex A is quite specific that endpoints showing difference or non-equivalence must be further analysed to investigate possible site x treatment interactions. This approach is fully in line with the oft-stated philosophy of the GMO Panel that the requirements of toxicological testing shall be considered on a case-by-case basis. Annex A has been amended to clarify some of these issues (see Annex B).

Some comments questioned why equivalence limits should be based on commercial varieties grown at the same sites when information on natural variation exists in sources such as the ILSI database. The GMO Panel and its statistics WG believe strongly that whilst it might be true that when a substantial database of commercial varieties has been established, future guidance might remove the necessity to include commercial varieties. However, that stage is nowhere near having been reached yet. It is essential that future implementations of suitable databases include detailed information on the particular variety concerned and a sufficient characterisation of the environments concerned to allow the elimination of major environmental differences in the comparison of GMO with commercial varieties.

Further questions asked why commercial varieties had to be integrated, as fully randomized and replicated treatments, into compositional field trials, and why the natural variability of varieties could not be estimated from unrandomized additional plots external to the field trial at a site, or even at other sites. The reason is that when commercial lines are included in the same experiment where the GMO is tested against the comparator(s) then data on commercial varieties are obtained in identical conditions to that of the GM and its comparator. This has the major advantage of eliminating uncontrollable confounding effects. The GMO Panel and its statistics WG affirm that randomization is a fundamental principle of good experimental design. Using information from unrandomized sources, as it was noted in some comments, would result in a biased estimate of the difference between the GMO and the commercial varieties. It would be completely unacceptable to place the entire basis for an equivalence test on an estimate of a difference that is biased.

In response to some comments received, the GMO Panel and its statistics WG have considered strategies to maximise the efficiency of trials on a given site, and have suggested designs by which the production of material for the comparative assessment of several different GM plants of the same crop species may be produced simultaneously at the same site and within the same field trial. This may be done by the placing of the different GM plants and their appropriate conventional counterpart(s) in the same randomized block, with some provisos. More details are given in Annex B.

There was a plethora of requests for a clearer description of how the equivalence limits should be calculated. In response to these the GMO Panel and its statistics WG have done further work. The final report of the GMO Panel statistics WG (to which Annex A and B makes reference) was amended to try to provide the needed clarification, also by supplying a worked example. The statistics WG recommendation for the estimation of equivalence limits is designed to effectively quantify the background variation between different varieties. Since each variety may be grown on a number of different sites (as long as these are appropriate), the estimated variation between varieties then takes automatically account of both genotypic variation and part of the full genotype x environment variation. The statistics WG has experimented with several formulae and has formulated guidance based on an estimate involving the standard error of the difference between the mean of the GM and of the commercial varieties. This formulation fulfils three important criteria:

i) The width of the equivalence interval is positively related to the degree of the measured genotype x environment interaction;

ii) GMO means fall within the equivalence limits with approximately the correct coverage (95%) under the null hypothesis that the GMO is exchangeable with any of the commercial varieties;

iii) It is easily implemented via the statistical mixed model in common statistical software (as illustrated now in the example given in the report).

There were a large number of responses that questioned the basis for comparison of the equivalence test. The draft for public consultation had been misunderstood as giving the false impression that the equivalence test was based somehow on the mean of the comparator. This is not so, and the GMO Panel and statistics WG have now provided clarification, both in the updated report of the statistcs WG and also within Annex B. The methodology for the equivalence test compares the mean of the GM with the mean of the commercial varieties. In fact, the comparator mean does not influence the result of the test. Probably what gave rise to the misunderstanding was the form of the graph that is the basis of the required presentation of results. On this graph, the value of the GM and of the equivalence limits are all related to a baseline from which the mean comparator value has previously been subtracted. Indeed, simple mathematics will convince that the mean value of the comparator has no effect whatsoever on the test of equivalence, since the relevant difference tested is: [mean(GM) - mean(comparator)] – [mean(commercial varieties) – mean(comparator)]. Similarly, the equivalence limits themselves, when plotted on the graph, are related to a baseline from which the mean comparator value has previously been subtracted, and so are also recalculated to subtract this value from their raw values, but solely in order to achieve a consistent scale for the graph.

Some comments requested clarification that Annex A was not intending to prevent the widespread practice of the use of more than one background germplasm for GMO and comparator(s) in order to better accommodate different environmental conditions. Clarification has now been provided.

There was some misapprehension in the comments that the choice of the minimum number of replicates at a site was driven by a technical desire to have a minimum number of degrees of freedom for error in an individual trial. In reality, the requirements are driven by the desire to have adequate replication and hence to ensure that each field trial at each site has sufficient power to give the Panel and the Member States confidence that unintended effects will be detected. Whilst the key statistical analysis for comparing GMO and comparator(s) is the analysis which averages across locations, a fundamental principle underpinning the previous Guidance was that replication should be sufficient at each site to allow an adequate stand-alone analysis at each of those sites. This is especially important in the frequent circumstances of treatment x site interactions when there are differences detected at some sites but not at others. That principle remains undiminished within the current report of the statistics WG and Annex B. Additionally, whilst it may be the case that coefficients of variation at the plot level for compositional trials are typically relatively low, this is not the case for all endpoints.

The GMO Panel and its statistics WG note with regret that, despite frequent emphasis on the need for statistical power calculations to guide replication levels in the previous food-feed (2006) Guidance, dossiers have only rarely included such studies. The result is that there is

little specific data available on which to base decisions concerning replication levels. Furthermore, in the interests of transparency it is important that a high level of confidence be given to the public on this issue. The GMO Panel and its statistics WG are of the opinion that the proposed guidance concerning replication at a site was satisfactory and required no amendment or clarification.

There were several comments on the need for data transformation. Data transformations may be used either to stabilise otherwise heterogeneous variance, or to change the scale on which effects are additive, or both. Statistically, the more important of these is additivity. The draft for public consultation issues no mandatory instructions to transform to logarithms, although the statistics WG expects that the great majority of endpoints will be. Indeed, in its research, it found that analysis with transformation generally provided equal or better results than analysis without. A logarithmic transformation has the advantage that differences may be expressed as percentage changes on a multiplicative scale. Here again, the GMO Panel and its statistics WG concluded that Annex A was satisfactory and required no amendment or clarification.

Annex B (and the report of the statistics WG) has been amended with clarifications on what should be the form of analysis for trials involving more than one comparator, and on how factors should be specified, as fixed or random, in the statistical mixed model.

**Summarised response to issues related to criteria for conventional counterpart selection and risk assessment of GM plants containing stacked transformation events**

This section responds, in a summarised form, to the main issues raised on the criteria for conventional counterpart selection and the risk assessment strategy of GM plants containing stacked transformation events, both described in the Annex A. Annex B provides a revision of Annex A, taking into account relevant comments received during the public consultation. In particular, in section 1.3.1 of Annex B the criteria are discussed regarding the choice of the conventional counterpart(s) and possibly additional comparators. Whereas in section 3.2 the approach is discussed for the risk assessment of GM plants containing stacked transformation events combined by conventional crossing. Additional clarifications for the risk assessment of GM plants containing stacked events are provided throughout Annex B as necessary and relevant in the various sections.

In general the proposed criteria for the selection of conventional counterparts and the approach for the risk assessment of GM plants containing stacked transformation events were well accepted and the number of comments received was limited.

Some comments requested clarifications on the criteria proposed for the selection of a suitable comparator(s). Other comments requested the possibility of using negative segregants as comparators in case non- GM conventional counterparts are not available. The GMO Panel has further detailed in Annex B the explanation of the properties that define a suitable comparator, and a description of the criteria to be followed for choice of comparator is provided in Annex B: '*in the case of vegetatively propagated crops, the conventional counterpart shall, in principle, be the non-GM isogenic variety used to generate the transgenic lines and with a history of safe use. In the case of crops that reproduce sexually, the conventional counterpart shall have a genetic background that is as close as possible to the GM plant and with a history of safe use'*.

The definition of comparator is in line with the requirements of Reg. (EC) 1829/2003 which states that comparators (or conventional counterparts) should be non-GMOs with a well-established history of safe use (see Article 2). It is also in line with the definition of suitable comparator provided by *Codex Alimentarius* (*Codex Alimentarius* Guidelines, 2003) where it is explained that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts. The ultimate objective of the comparative assessment is the detection of both intended and unintended effects. In particular, the detection of possible unintended effects and their consequences needs a careful systematic screening of the GM plants compared to their traditional counterparts. In this frame, any comparator not fulfilling the requirements listed above - namely a comparable genetic background, a history of safe use and a non-GM status - cannot be considered as appropriate for the comparative assessment. Since a negative segregant is derived from a line that has been genetically modified, although it has a comparable genetic background, may still carry possible unintended effects and does not have a history of safe use. However negative segregants may in addition and case-by-case provide useful information.

In response to other comments, the GMO Panel has further elaborated guidance and clarified the requirements for performance of field trials in case of herbicide tolerant crops.

In response to other comments, the GMO Panel has further elaborated on the risk assessment strategy recommended in cases where an appropriate non-GM comparator cannot be identified. The Panel recommends that a full risk-assessment should be performed by the applicant in order to provide a comprehensive safety and nutritional assessment of the GM crop derived food/feed *per se*.

**Summarised responses to comments related to toxicological and nutritional assessment.**

This section responds, in a summarised form, to the main issues raised on the approach undertaken by the GMO Panel regarding the toxicological and nutritional assessment. This approach is described in Annex A. Annex B provides a revision of Annex A, taking into account most of these issues in particular in its sections 1.4, 1.6 and 1.7.

There were several comments on the need for testing of whole GM food/feed using a 90- days rodent animal feeding trial routinely. The GMO Panel would not recommend this approach. This issue has been comprehensively addressed in the Report of the EFSA GMO Panel WG on Animal Feeding Trials (Food and Chemical Toxicology, Vol. 46, Supplement 1, March 2008). The EFSA GMO Panel is of the opinion that performance of a 90-days rodent feeding trial study with whole GM food/feed can be used for reassurance of the performed risk assessment. In addition, it should be performed in case of extensive alterations in the composition of the GM food/feed or in case of indications for the occurrence of unintended effects based on evaluation of molecular, biochemical, compositional, and phenotypic and agronomical aspects. The limited sensitivity and specificity of the study prevents it from being used as the main test in the safety assessment. Thus, a case-by-case approach is recommended. In Annex B the conditions for carrying out feeding trials in rodents are explained more explicitly.

There were some comments regarding the methodology to be applied in a 90-days rodent feeding trial study. The GMO Panel notes that it is important to have at least two dose levels in the 90-day rodent feeding trial study in order to assess the toxicological relevance of any

observed difference(s) between groups. This issue and other issues related to this topic have been comprehensively addressed in the Report of the EFSA GMO Panel WG on Animal Feeding Trials (Food and Chemical Toxicology, Vol. 46, Supplement 1, March 2008). The report recommended that laboratory animal feeding studies with defined single substances should be conducted according to the OECD Guidelines for the Testing of Chemicals (OECD Test Guidelines) and in compliance with the principles of Good Laboratory Practice (GLP). Regarding novel foods and feeds, the Report recommended an adaptation of the existing OECD methods for subchronic toxicity testing. In particular of TG 408 *Repeated Dose 90-day Oral Toxicity Study in Rodents*.

Some comments requested a clear definition of the history of safe use of the conventional counterpart and the new protein. Regarding the conventional comparator and in line with the Codex guidelines (23.d), the GMO Panel considers it necessary to have information on the history of safe use of the host plants for consumption as food and feed. There is indeed no currently generally accepted definition of the term 'history of safe consumption'.

Regarding the newly expressed protein, at least the exposure levels resulting from previous consumption of foods containing this protein should be considered and information on previous consumption of the protein should be provided.

There were a large number of responses that questioned the opinion of the GMO Panel that the acute oral toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of GM food/feed and it was suggested to use an acute toxicity test for the identification of the NOAEL. This test is primarily used for screening of acute toxic effects of chemical compounds, and is of little value for the assessment of potential adverse effects due to regular (chronic) consumption of foods. Unless reliable information is provided which demonstrates the safety of the newly expressed protein, the safety assessment of proteins with no history of safe use (for consumption as food) should normally include a repeated-dose toxicity test (normally 28 days) and not rely on acute toxicity testing. Depending on the results obtained from such test, further testing may be necessary.

A few comments raised the concern if the test protocols tabled in the Annex A are obligatory for the risk assessment of GM plants. The GMO Panel would like to clarify that in case specific aspects should be investigated these protocols should be applied.

Questions were raised on the potential increased toxicity and/or allergenicity to humans and animals or modified nutritional value due to consumption of stacked events. Potential adverse effects may arise from possible interactions between the newly expressed proteins, new metabolites and original plant constituents. The risk assessment of stacked events requires a case-by-case approach focused on the identification of potential interactions between the indicated constituents. If the potential for adverse interactions is identified, animal feeding trials with the whole GM food/feed are required as explained in the section 1.4.4.1 of Annex B.

Several comments were questioning the relevance of the guidance of the GMO Panel (Annex A) and consequently of Annex B, for the risk assessment of GM foods with altered nutritional or medical properties and the need for human studies. The toxicological and nutritional assessment of GM plants with an altered level of specific nutrients and GM plants intended

to provide additional health benefits is addressed in Annex B (in particular in Part II, sections 1.4.2, 1.4.3, 1.6 and 3.)

For the risk assessment of GM plants with an altered level of specific nutrients and GM plants intended to provide health benefits, existing reference values for acceptable or tolerable levels of intake of the specific substance(s), e.g. the Acceptable Daily Intake (ADI) or Tolerable Upper Intake Level (UL), should be taken into account (see Part II, section 3 in the Annex B). If no such value has been derived, information for the toxicological and nutritional assessment has to be provided according to Part II, sections 1.4.2. and 1.4.3/ 1.6 of Annex B, respectively. This may include comprehensive toxicological testing of the single substances, including studies in humans as well as bioavailability studies. Health, nutritional status and dietary practices of specific population(s) anticipated to consume the food should be considered in the assessment (see Part II, section 1.6.1 of Annex B). The Panel will consider the need for new guidance on this subject based on the experience from the evaluation of new products.

A clear distinction should be made between GM plants with an altered level of specific nutrients and GM plants intended to provide health benefits on the one hand and GM plants expressing substances intended to be used in medicinal products on the other hand. The risk assessment of the latter is described in the Guidance Document for the risk assessment of GM plants used for non-food/feed purposes (EFSA, 2008). It is emphasized that the safety and efficacy evaluation of the medicinal product as such is not within EFSA's remit.

**Concluding remarks**

The GMO Panel acknowledges the usefulness and quality of a large number of comments and would like to thank all stakeholders for their interest and input to its current and future work.

***Acknowledgments*:** EFSA would like to thank the Working Groups of the GMO Panel on Molecular Characterisation and Food and Feed safety as well as its staff Anna Christodoulidou, Zoltán Divéki and Claudia Paoletti for the preparation of this document.

**Appendix:**

The text below is from the EFSA website of the public consultation:

**Updated guidance document of the Scientific Panel on Genetically Modified Organisms (GMO) for the risk assessment of genetically modified plants and derived food and feed**

Deadline: 21 September 2008

EFSA's GMO Panel regularly reviews its guidance to take account of scientific developments and of experience gained through the risk assessment process. When doing so the Panel, in line with EFSA's policy on openness and transparency, and in order to allow the scientific community and stakeholders to comment on its work, consults the public on key issues.

In 2008 the Panel updated its guidance document, originally adopted in 2004, in the light of several years of experience in the risk assessment of GMO applications and of the outcome of the Panels' self-tasking activities. Currently the updated guidance document forms the basis for the establishment of legal EC Guidelines by the Commission and the Member States. In the frame of enhancing that process, the outcome of this consultation will be directly forwarded to the Commission and the Member States. EFSA will publish an evaluation report on the comments received and be consulted prior to the adoption of the legal framework.

Interested parties are invited submit written comments by **21 September 2008**.

Please exclusively use the electronic template provided with the documents to **submit comments** and refer to the line numbering. Comments submitted by email or via surface mail cannot be taken into account.

**Note:** No consultation is launched for the sections of the guidance concerning environmental risk assessment. Additional consultation on these sections is foreseen after their update on issues such as assessing potential long-term environmental effects of GMO cultivation, following a mandate from the Commission's DG Environment and the outcome of activities that EFSA has initiated on assessing potential risks to non-target organisms by traits such as insect-resistance in GM plants.

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# ANNEX A

# UPDATED GUIDANCE DOCUMENT FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED

# ANNEX B

# ANSWER FROM EFSA TO THE CONSULTATION OF DG SANCO ON RULES FOR APPLICANTS FOR THE PREPARATION AND PRESENTATION OF APPLICATIONS TO BE SUBMITTED UNDER REGULATION (EC) NO 1829/2003 ON GM FOOD AND FEED

# ANNEX C

# LIST OF COMMENTS RECEIVED DURING THE PUBLIC CONSULTATION ON THE UPDATED GUIDANCE DOCUMENT FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED, DRAFT DOCUMENT ADOPTED IN MAY 2008