Risk Assessment of "stacked events"

Untersuchungen zur Risiko-abschätzung von "Stacked Events"

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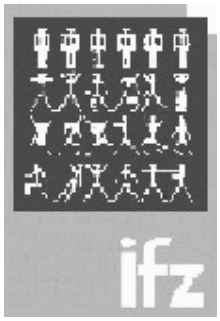

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Summary

In recent years an increasing number of genetically modified plants (GMPs) which combine two or more transgenic traits, so called "stacked events", have been notified under Regulation (EC) No. 1829/2003 as well as Directive 2001/18/EC. These hybrids were derived from GMPs by breeding methods without additional genetic modification. Based on EU legislation, these kinds of GMPs have to undergo a standard authorisation procedure including risk assessment.

Recent discussions about applications for "stacked events" and the respective opinions delivered by the European Food Safety Authority (EFSA) have highlighted the controversy among risk assessors and among Member States about the particular risk assessment requirements for applications for this type of GMPs. Most of the controversy is about requirements in addition to the data submitted for the parental GM events. Very recently, in mid 2006, EFSA published a Draft Guidance Document on this topic and launched a public consultation.
Against this background, this study aims at identifying key issues for the risk assessment specific to "stacked events" and investigates how these issues are considered in practice. Based on these experiences the study identifies open questions and the need for further clarification and research.

The analysis draws on two case studies, GM maize lines 1507 x NK603 and MON863 x MON810. For these "stacked events" applications have been submitted according to Regulation (EC) 1829/2003 and Directive 2001/18/EC, with the risk assessment having been evaluated in the opinions delivered by EFSA and by Member State authorities. Furthermore a comparison of specific guidance documents is included.
Some of the key issues identified appear to be less controversial or not controversial at all:

- Risk assessment of "stacked events" can draw on the assessment of parental GMPs and should be based on the results of such assessments.
- Requirement for a molecular characterisation in order to confirm the preservation of the inserted traits and to compare the expression of transgenes between parental GM events and the "stacked event".
- Comparative analysis of the "stacked event" including a standard set of compositional and agronomic parameters.
- Need to consider any potential interaction of combined GM traits in the "stacked events".

On the other hand, some issues are still contested and require further clarification, discussion and research:

- Can the methods used for molecular characterisation be considered sufficiently precise?
- Should the parental GM events be included in the comparative analysis of plant compounds, agronomic traits, and expression of the transgenes along other comparator lines?
- How could the potential interaction of traits be assessed and at what level (genetic, protein, metabolic)?
- What would trigger whole food toxicity studies?
- Are the parameters investigated for "stacked events" suitable to identify specific characteristics, which need to be addressed in the environmental risk assessment?

The latter set of questions refers to issues that are neither appropriately addressed in the risk assessment dossiers nor analysed in the guidance documents. Furthermore the proposed EFSA guidance on the risk assessment of stacked events does not add much more clarity to the current general EFSA guidance on assessing GMOs.
In the view of the authors of this report, these issues should be further discussed by a broader range of experts. The objective should be to provide a more detailed and scientifically more robust guidance document, which would be of relevance for applicants, risk assessors and regulatory bodies.

Zusammenfassung

In den letzten Jahren wurde in der EU eine steigende Zahl von genetisch veränderten Pflanzen (GVP) mit kombinierten Eigenschaften zur Zulassung nach der Richtlinie 2001/18/EG bzw. der Verordnung (EG) No. 1829/2003 angemeldet. Diese so genannten "stacked events" GVP werden durch Kreuzung von zwei oder mehr GVP hergestellt und enthalten damit eine Neukombination von Eigenschaften, ohne dass dazu eine weitere genetische Veränderung nötig wäre. Nach den geltenden Vorschriften müssen diese "stacked events" ein Standard-Zulassungsverfahren durchlaufen und dabei einer Risikoabschätzung hinsichtlich ihrer möglichen Auswirkungen auf Gesundheit und Umwelt unterzogen werden.

Kontroversen über die Zulassung von "stacked events" und insbesondere über die Erfordernisse für die Risikoabschätzung bei "stacked events", haben einen hohen Klärungsbedarf angezeigt. Im Sommer 2006 wurde zu diesem Thema seitens der europäischen Behörde für Lebensmittelsicherheit (EFSA) ein Entwurf für Richtlinien für die Risikoabschätzung bei "stacked events" veröffentlicht und ein Konsultationsverfahren zu diesem Entwurf eingeleitet.
In diesem Kontext wurde die vorliegende Studie durchgeführt, um Schlüsselelemente für die Risikoabschätzung von "stacked events" zu identifizieren und zu untersuchen, wie diese in der regulatorischen Praxis bei der Risikoabschätzung von bestimmten GVP berücksichtigt werden.

Die regulatorische Praxis wurde anhand zweier Beispiele, der genetisch veränderten Maislinien 1507 x NK603 and MON863 x MON810, untersucht. Für diese „stacked events" wurden Zulassungsanträge auf Basis der Regelungen in der Richtlinie 2001/18/EG und der Verordnung (EG) 1829/2003 gestellt. Die Zulassungsanträge wurden zudem bereits von der EFSA und den zuständigen Behörden der EU-Mitgliedsländer begutachtet. Die Analyse der Fallbeispiele beleuchtet damit die praktischen Schwierigkeiten bei der Risikoabschätzung derartiger GVP. Zudem wurden eine Reihe publizierter Dokumente zum Thema, darunter die verfügbaren Richtliniendokumente in die Untersuchung einbezogen.

Die Analyse hat ergeben, dass hinsichtlich einer Reihe von Grundsätzen für wesentliche Aspekte der Risikoabschätzung für "stacked events" weitgehende Übereinstimmung besteht:

- Die Risikoabschätzung für "stacked events" soll auf der Risikoabschätzung für die jeweiligen GVP-Linien aufbauen, die für die Herstellung des "stacked events" verwendet wurden
- Es soll durch molekularbiologische Untersuchungen gezeigt werden, dass die genetischen Veränderungen in den "stacked events" den in den Ausgangslinien vorkommenden Modifikationen entsprechen. Zusätzlich sollen die von den genetischen Veränderungen bewirkten Eigenschaften vergleichend bei den "stacked events" und den Ausgangslinien überprüft werden.
- Durch eine vergleichende Analyse der "stacked events" mit konventionellen Maissorten sollen die inhaltsstoffliche Zusammensetzung der "stacked events", sowie die agronomischen Eigenschaften überprüft werden.
- Wesentlich ist es, alle Effekte, die sich durch die spezielle Kombination der eingebrachten Eigenschaften in den "stacked events" ergeben, zu untersuchen:

z.B. spezifische Gesundheits- und Umweltwirkungen, welche durch diese Kombination von Eigenschaften ausgelöst werden.

Andererseits zeigen die Ergebnisse, dass bezüglich einer Reihe von Fragen noch erheblicher Konkretisierungsbedarf und Forschungsbedarf besteht. Klärungsbedürftig sind unter anderen folgende Punkte:

- Sind die bei der molekulargenetischen Charakterisierung von "stacked events" verwendeten Methoden geeignet, geringfügige aber eventuell relevante Veränderungen mit hinreichender Sicherheit nachzuweisen?
- Sollten die Ausgangs-GVP in der vergleichenden Analyse für "stacked events" in den Feldversuchen als Kontrollen jeweils miterfasst werden?
- Wie können mögliche nachteilige additive oder synergistsche Wirkungen der veränderten Eigenschaften erfasst werden und auf welcher Ebene (DNA, Protein, Stoffwechsel) soll das geschehen?
- In welchen Fällen sind weiterführende Untersuchungen z.B. Studien zur subchronischen Toxizität der gentechnisch veränderten Lebensmittel notwendig?
- Welche Informationen müssen vorgelegt werden, um konkret entscheiden zu können, welche spezifischen Eigenschaften im Hinblick auf Umweltwirkungen weiter zu untersuchen und zu bewerten sind?

Diese Fragen lassen sich weder aus den Antragsunterlagen noch in den bisher vorliegenden Leitlinien klären. Auch der vorgeschlagene Richtlinienentwurf der EFSA weist diesbezüglich erheblichen Konkretisierungsbedarf auf. Die weitere Diskussion sollte auf diese Fragen eingehen und einen größeren Kreis von Fachleuten einbeziehen. Ziel dieser Diskussion sollte die Erarbeitung von wissenschaftlich tragfähigen und hinreichend konkreten Leitlinien für die Risikoabschätzung von "stacked events" sein, was im Sinne von Antragstellern, Behörden und Risikobewertern sein sollte.

Introduction

Since 2004 the number of applications for commercial use of genetically modified (GM) plants in the European Union has significantly increased. Several recent developments accompany the process of commercial introduction of GM crops in Europe. Cultivation of GM maize varieties with single modified traits is gradually increasing in some parts of Europe.
Also, an increasing number of GM crops have been developed and notified that combine two or more transgenic traits originally present in different parental GM events, thus producing so called "stacked events", also designated as "stacked products", "breeding stacks" or "pyramided events" (De Schrijver et al. 2006).

"Stacked events" can be considered different from parental GM events for a number of reasons:

- "Stacked events" do contain a new combination of transgenic traits and genetic backgrounds derived from single event GMOs.
- In contrast to parental GM events they are not constructed by direct genetic modification. In the case of "stacked events", the above mentioned new combination is produced by crossing different parental GM events.
- From an EU regulatory perspective "stacked events" differ from GM hybrids, which are derived from one parental GM event by crossing with a non-GM variety. "Stacked events" are currently considered as new GMOs, which need to undergo risk assessment similar to parental GM events and obtain regulatory approval, before they can be placed on the market (EC 2003b, EFSA 2004b).
- Given the experience from recent risk assessments of "stacked events" the particular data requirements are contested among national competent authorities (CAs), as well as between national CAs and EFSA.

From a regulatory perspective "stacked events" are currently considered in the EU as new GMOs, which need to undergo risk assessment similar to parental GM events and obtain regulatory approval, before they can be put on the market (EC 2003b, EFSA 2004b).
Considering the developments described above it is important to clarify the requirements for the risk assessment for this type of GMOs. In the absence of specific guidance for stacked events, the general guidelines for risk assessment according to Dir. 2001/18/EC and Reg. (EC) No. 1829/2003 apply to "stacked events" as well.

Given the increasing number of such "stacked events" entering the application processes (see Table 4, Annex 1) and the limited experience in handling such applications, specific guidance is required for applicants and regulatory bodies involved in the authorisation processes, as well as for Scientific Committees involved in the risk assessment of GMOs. However, few specific guidance documents are available and none of them has been agreed so far between the various stakeholders in the process (De Schrijver et al. 2006). Furthermore, for assessing specific issues such as interactions between individual transgenic traits the scientific basis is complex and not entirely clear.

Different views on the risk assessment of "stacked events" have emerged (summarised in De Schrijver et al. 2006). Comments submitted to EFSA and the GMO Panel of EFSA criticised the risk assessment conducted for some stacked

event GM crops. This is well documented in the comments of Member State CAs on specific applications and in a response from ACRE, the Scientific Committee of the UK CA. Some Member State CAs asked for a more comprehensive risk assessment for certain stacked events, whereas ACRE could not see any generic reasons for carrying out additional evaluations other than those included in the risk assessment on the parental GM events (ACRE 2004).

The following aspects appear to be crucial for the risk assessment:

- The characterisation of any modifications in the "stacked event" compared to parental GM events.
- The phenotypic characterisation of the "stacked event", which depends on the new combination of traits and genetic backgrounds. A thorough characterisation is required to identify any differences of the stacked event to the conventional counterparts and the respective parental GM varieties, which would require further risk assessment.
- Any effects resulting from additive and synergistic or antagonistic interactions between modified traits and genetic backgrounds. Here the risk assessment could start with an analysis of which effects of the stacked event were already assessed in parental GM events and which effects need to be considered specifically for the stacked events.
  Examples of such effects to be assessed for "stacked events" are:
  altered potential for adverse effects on target organisms and non-target organisms; altered environmental exposure with respect to the conditions of intended use, specifically under conditions of commercial crop production; effects of specific cultivation management with respect to target and non-target organisms and considering effects on biodiversity at all trophic levels.
- Also any monitoring activities intended for "stacked events" need to consider the above mentioned aspects and be tailored to the specific characteristics of the „stacked event".

In this report we identify and investigate approaches to the assessment of "stacked events" by analysing specific guidance documents (Chapter 4) and data provided in selected EU applications for placing specific "stacked events" on the market as GM crops (Chapter 5). The analysis aims to identify differences and similarities in order to highlight areas of dissent and inconsistencies that would require further attention. A draft guidance document issued recently on the risk assessment of plants containing genetic modification events combined by crossing (EFSA 2006a) had been analysed and commented in detail during an earlier phase of this study. This draft had been published for public consultation in mid 2006 (see Annex 4). The comments of the authors directed at EFSA are annexed to this report.
In a concluding chapter key issues of the risk assessment of "stacked events" are discussed (Chapter 6).

Guidance Documents

This chapter provides a brief overview of the risk assessment requirements for "stacked events" proposed in guidance documents and in the scientific literature. "Stacked events" are briefly addressed in the EFSA general Guidance for GM plants (EFSA 2004b) and some issues concerning "stacked events" have been

discussed in several international contexts, e.g. the Codex Alimentarius Commission (Codex 2006) and on Member States level (e.g. ACRE 2004).

Until recently specific guidance on risk assessment strategies for "stacked events" were restricted to EuropaBio, the European biotechnology industry association (EuropaBio 2005). In mid 2006 a draft guidance document was published by EFSA. With the exception of a very recent article of De Schrijver et al. (2006) there is little discussion of these issues in the scientific literature.

The above documents outline risk assessment requirements from very different perspectives (EFSA Scientific Panel, industry association, scientists). Thus a comparison of the proposals and approaches described in these documents was undertaken to identify potential areas of consent and dissent.
The proposals provided in the two guidance documents and in the article of De Schrijver et al. (2006) were compared in terms of their overall approach and detailed requirements. Table 5 in Annex 2 provides an overview.

Scope of the analysed documents

In all documents only the case where two or more GM events that are combined by conventional breeding techniques such as crossing are considered. By contrast, the OECD definition of stacked transformation events includes re-transformation (OECD 2002).
There is agreement between different documents (EuropaBio 2005, De Schrijver et al. 2006) that retransformed GMP varieties (re-transformation of existing GM lines with a different transgenic construct as well) have to be assessed like single GM events according to existing regulations and guidelines (EC 2001, 2002, 2003a, 2003b; EFSA 2004b), since they meet the definition of the EU Scientific Steering Committee that the risk assessment process concentrates on the outcome of transformation processes (EC 2003b).
Furthermore, all three documents focus on such "stacked events" the parental GM events of which have already been assessed and received a favourable opinion. In case one or more of the GM events had not been assessed before, an assessment of these particular events would be necessary according to the guidelines established for single traits (EFSA 2004b).

Molecular characterisation

The EFSA Draft Guidance requires a demonstration of the **intactness of the inserted traits** in the "stacked events" by appropriate molecular approaches and in comparison to the parental GM events. Southern blots and PCR analyses spanning the inserts and flanking regions are proposed as suitable methods. Likewise De Schrijver et al. (2006) suggest Southern blot analyses to prove the correct transfer of transgenic traits to GM "stacked events". Whether this method is suitable to provide the necessary basic molecular information to confirm the presence of transgenic genetic elements against different genetic backgrounds in GMPs which have been produced by conventional breeding is under discussion. Still, the importance of confirming the overall structure of inserts derived from single event GMPs including flanking regions is stressed.
De Schrijver et al. (2006) do not provide recommendations on how possible point mutations, deletions and other re-arrangements in the stacked event DNA or minor modifications of the inserts should be detected. It is noted that the suggested correlation with data on the expression of inserted traits and compositional analysis can only detect changes which would directly affect the expression of the assessed compounds.
EuropaBio suggests restricting the molecular characterisation to fingerprint analysis by Southern Blot (EuropaBio 2005).

With regard to **phenotypic stability** EFSA suggests that confirmation should be established of the transgenic traits remaining unchanged in the specific "stacked event" GMP. Any significant changes in the expression of traits and in the phenotype of the "stacked events" in comparison to parental lines should raise concern and prompt further assessment during risk assessment, including the environmental risk assessment (EFSA 2006a). Likewise EuropaBio and De Schrijver et al. (2006) stress the importance of comparing expression levels of "stacked events" to the respective levels in parental GM events, without specifying any experimental design and duration of assessments. However, robust data are necessary to identify whether the combined presence of

transgenes influences expression levels, e.g. by silencing effects (Fagard & Vaucheret 2000 in De Schrijver et al. 2006).

EFSA generally requests an assessment of potential interactions between traits combined in the "stacked events" (including the effects on the expression of traits as mentioned above). Such assessments are considered relevant for an evaluation of possible health or environmental effects associated with "stacked events". With respect to the evaluation of segregants from "stacked events", it is proposed that individual properties and characteristics of parental GM events should be considered in this context (EFSA 2006a).

Guidance on how to assess the **genetic stability** of inserted traits over several generations is not given explicitly, neither by EFSA nor by EuropaBio. EFSA only refers to the required data of the expression analysis, which might indicate possible stability issues (EFSA 2006a, EuropaBio 2005).

Environmental risk assessment

Both EFSA (2006) and De Schrijver et al. (2006) mention that the environmental risk assessment has to take into account information from the environmental evaluation of the parental GM events and all information that is valid also for the "stacked event". With this information as a starting point the assessment should focus on possible environmental effects resulting from aspects of the „stacked event", that might trigger specific impacts on the environment.

EFSA specifies interactions between genes and gene products which might lead to changes in the physiology of the "stacked events" or related species with potential modifications of the ecological behaviour (EFSA 2006a). As to which specific effects need to be considered due to the specific nature of the "stacked events", EFSA refers to the respective issues included in the EFSA guidance document (EFSA 2004b) and to a non-exhaustive list of such issues (altered potential for target effects and related consequences such as development of resistances, enhanced toxicity to non-target organisms including changes in the range of organisms affected or altered fitness of the stacked event or related plants which might acquire the inserted traits, specific capacities for gene transmission and effects on microbial diversity and geochemistry).
Regarding specific effects associated with "stacked events", De Schrijver et al. (2006) highlight effects by traits that are similar or potentially synergistic regarding their mode of action. Examples of such effects due to the interaction of traits are given with regard to applications involving insect resistance and herbicide tolerance. Combined effects of Bt-toxins in "stacked events" and enhanced potential for cross-resistance development are mentioned. Furthermore the enhanced potential of crops with several resistance traits to affect persistence and invasiveness of GMOs and to affect biodiversity due to changed agricultural practices is considered. An assessment of these potential effects should be done by field testing before commercialisation and post-market monitoring (De Schrijver et al. 2006).
EuropaBio does not give specific recommendations on how to assess environmental effects other than evaluating the agronomic properties of the "stacked event". This should be done for cultivation applications with trials in Europe at sites representative of the range of agricultural environments typical of the respective type of crop. The number of sites is not specified in this context (EuropaBio 2005).

Compositional analysis

With respect to compositional analysis both the EuropaBio Guidance and the EFSA Draft Guidance suggest reducing the number of seasons to one compared to the two seasons normally required. EuropaBio suggests conducting field trials at four sites, whereas EFSA does not specify the number of sites. Geographical representativeness of field trial locations is considered important in both documents. Concerning the controls used in field trials, EFSA does not explicitly mention parental GM events as comparators, whereas EuropaBio and De Schrijver et al. (2006) consider parental GM events optional but not additional comparators. Risk assessment according to the EuropaBio proposal requires agronomic data of the stacked event, whereas the EFSA Draft does not specify any specific requirements.

Toxicity, allergenicity and nutritional assessment

Whole food toxicity studies are considered to be a specific rather than standard requirement in all three guidance documents. While EFSA does not specify what would trigger such studies, De Schrijver et al. (2006) put emphasis on altered expressions of the transgene and on molecular stability. Similarly, EuropaBio considers such studies necessary if possible changes to the protein mode of action can be expected and if "appropriate animal feeding studies" are not available. ACRE (2004), however, does not consider any additional studies necessary at all if the parental GM events are properly assessed.
No specific guidance is given on how to assess possible interactions of introduced proteins. De Schrijver et al. (2006) highlighted the importance of this issue and that there are no tests available.

The potential allergenic properties of whole plants are mentioned by De Schrijver et al. (2006). However they do not conclude that specific tests or data are required for an assessment of these effects.

Case studies

This chapter presents a case-specific analysis of applications for "stacked events". The individual applications were selected as described in the first section (Section 5.1)

Selection of applications for analysis

Applications for "stacked events" relevant for the analysis to be conducted were submitted under two different pieces of European legislation:

1) Applications for placing "stacked events" on the market according to Dir. 2001/18/EC (Part C),
2) Applications according to Reg. (EC) No. 1829/2003

Applications according to Dir. 2001/18/EC (Part C) are processed by the Competent Authority of the Member State where the application was filed. The procedure involves the other Member States and European Community bodies for final authorisation. A scientific opinion on the risk assessment has to be delivered

by the respective scientific committee (formerly Scientific Committee on Plants; currently EFSA GMO Panel).

Alternatively applications for placing "stacked events" on the market as food and/or feed including cultivation of GMOs are filed according to Reg. (EC) No. 1829/2003. They are processed in a more centralised way with the EFSA as the central authority in charge which considers comments of Member States authorities.

Recently there has been a tendency of filing applications under Reg. (EC) No. 1829/2003 with a broad scope (covering food and feed use, import and processing, and cultivation) according to the one-door-one-key principle. In such cases an environmental risk assessment according to the relevant provisions of Dir. 2001/18/EC is necessary, with a Member State Competent Authority responsible for this evaluation which provides an important input for the scientific panel of EFSA when drawing up an overall opinion concerning risk assessment.

As regards both types of application (according to Dir. 2001/18/EC as well as to Reg. (EC) No. 1829/2003), most of the dossiers on stacked events that have reached an advanced phase of the application process or received authorization are dossiers whose scope does not include cultivation and is limited to the importation and processing of GMOs. This restricts the range of dossiers available for an analysis which includes a comprehensive environmental risk assessment as well.
An overview of applications for "stacked events" submitted according to Reg. (EC) No. 1829/2003 as of December 2006 is presented in Table 4 of Annex 1 to this report.

The applications analysed in this report should be representative of the current situation regarding stacked event crops and the analysis should provide an insight into how the risk assessment of "stacked events" is conducted. The relevant criteria for the selection of appropriate applications are listed below (Table 1).

Table 1: Criteria for the selection of applications for a comparative analysis of case studies

Criteria	**Available Cases**
Type of crop	• Maize • Oilseed rape • Cotton
Type of inserted traits	• Insect resistance • Herbicide tolerance • Changed composition
Combination of inserted traits	• Same type of traits • Mixed combination of traits
Legal basis of application	• Dir. 2001/18/EC • Reg. (EC) No. 1829/2003
Scope of application	• Import and processing • Food and/or feed use • Cultivation
Status of application	• Valid applications • Applications with overall risk assessment
Relevance for Austria	• Crops cultivated

	• Potentially intended use

The selection of applications was based on the following considerations regarding the criteria listed in the above table:

- Maize "stacked events" were selected, based on the importance of maize as a crop of high importance for developing "stacked events"
- Insect resistance and herbicide tolerance are of prime importance and were therefore selected.
- Both types of trait combinations (different traits of same type, different traits) should be analysed.
- Selected applications should be representative of both types of legislation (Dir. 2001/18/EC, Reg. (EC) No. 1829/2003).
- Applications whose scope includes cultivation were preferred where possible; otherwise applications for import and processing, food and/or feed use were analysed.
- Based on the relevance for Austria, maize "stacked events" were selected, maize being an important crop in Austria and GM maize lines being regarded as potentially interesting at the international level.

The following table (Table 2) summarizes applications for maize stacked events according to Reg. (EC) No. 1289/2003 (whose scope includes cultivation) and according to Dir. 2001/18/EC (Part C).

Table 2: Maize stacked event applications considered for analysis

Application	Trait/Species	Scope of application	Applicant	Status
Reg. (EC) No. 1829/2003 **EFSA/GMO/UK/2005/17** ERA CA: Spain	1507 x NK603 maize	Food, feed Import and processing **Cultivation**	Pioneer Hi-Bred/ Mycogen Seeds	Valid application
Reg. (EC) No. 1829/2003 **EFSA/GMO/NL/2005/26** ERA CA: France	NK603 x MON810 maize	**Cultivation**	Monsanto	Under completeness check
Reg. (EC) No. 1829/2003 **EFSA/GMO/NL/2005/28**	1507 x 59122 maize	Food, feed Import and processing **Cultivation**	Dow AgroSciences	Under completeness check
Reg. (EC) No. 1829/2003 **EFSA/GMO/UK/2006/29**	59122 x NK603 maize	Food, feed Import and processing **Cultivation**	Pioneer Hi-Bred	Under completeness check
Reg. (EC) No. 1829/2003 **EFSA/GMO/UK/2006/30**	59122 x 1507 x NK603 maize	Food, feed Import and processing **Cultivation**	Pioneer Hi-Bred	Under completeness check
Dir. 2001/18/EC **C/DE/02/9**	MON863 and MON863 x MON810 maize	Import and processing	Monsanto	Pending
Dir. 2001/18/EC **C/GB/02/M3/03**	NK603 x MON810 maize	Import and processing	Monsanto	Pending

From this list the applications for **GM maize 1507 x NK603** and for **GM maize MON863 x MON810** were selected for analysis.

GM maize 1507 x NK603:

- The "stacked event" comprises a combination of insect resistance (due to an introduced Bt-toxin) and herbicide tolerance based on two different traits (to the non selective herbicides Glyphosate or Glufosinate-ammonium)
- Application according to Reg. (EC) No. 1829/2003 with a comprehensive scope including cultivation and a separate dossier with a scope excluding cultivation are pending. Only the latter application has yet received a scientific opinion on risk assessment from the EFSA GMO Panel.
  For an analysis both applications have been considered. Wherever different data are included in the dossiers, the respective sources are indicated in the following chapters.

GM maize MON863 x MON810:

- GM maize MON863 x MON810 is a combination of two different insect resistance traits, representative of such combinations.

- Applications are pending according to Dir. 2001/18/EC (Part C) for import, processing and feed use and under Reg. (EC) No. 1829/2003 for food and feed uses.

It should be noted that in the future other combinations of traits are likely to be notified, which may present different problems in the risk assessment. GMOs with modified metabolic pathways resulting in a changed composition, or GMOs modified to increase resistance to certain biotic and abiotic stresses are examples of such applications.

Maize 1507 x NK603

General aspects

The application for authorisation of 1507 x NK603 maize was submitted in 2005 by Pioneer Hi-Bred International and Mycogen Seeds according to Regulation (EC) No 1829/2003.
The scope of the application is use of maize 1507 x NK603 for food and feed purposes, food and feed containing, consisting of or produced from 1507 x NK603 maize and cultivation of GM maize 1507 x NK603.
The status of the dossier is that of a valid application after completion of the completeness check by EFSA. The application was reviewed by Member State Authorities. However at the time this report was produced no opinion was delivered by the EFSA GMO panel.
The GMO Panel is assessing 1507 x NK603 maize for the mentioned uses according to the principles laid down in the general guidance document for risk assessment of GM Plants and derived food and feed by the GMO Panel (EFSA 2004b).

GM maize 1507 x NK603 was developed by combining single maize events 1507 harbouring *cry1F* and *pat* genes and NK603 harbouring the *cp4 epsps* transgene. The resulting stacked event GM Plant is therefore exhibiting resistance to lepidopteran insects and pests due to the CRY1F-Toxin, and tolerance to the herbicides Glufosinate ammonium and Glyphosate due to the expression of PAT and EPSPS proteins respectively.

The single events 1507 and NK603 as well as the 1507 x NK603 maize have been assessed earlier. Maize 1507 was previously assessed under Dir. 2001/18/EC Part C for import and processing, as well as import, feed and industrial processing and cultivation and for food use according to Reg. (EC) No. 1829/2003. According to the assessment it had been authorised for import, processing and feed use under Dir. 2001/18/EC and for food use under Reg. (EC) No. 1829/2003. NK603 was assessed and authorised according to Dir. 2001/18/EC and for use for food and food ingredients under Reg. (EC) No. 1829/2003.

GM maize 1507 x NK603 was assessed by the EFSA GMO Panel with reference to food and feed use but without cultivation and received a favourable opinion (EFSA 2006a). The GMO Panel considers that this maize is unlikely to have any adverse effect on human and animal health or the environment in the context of its intended uses. A number of Member States have commented on the applications and expressed their concern about the initial assessment.

Risk assessment according to the applicant

No new modification was introduced into GM maize 1507 x NK603, but the single events 1507 and NK603 were combined by crossing the respective inbred lines. Accordingly the applicants describe most of the traits and characteristics of the "stacked event" as being the same as those of the parental GM events used in production of GM maize 1507 x NK603.

Molecular Characterisation

Specific analyses have been conducted concerning the **copy number, structure and organisation of the inserts** in GM maize 1507 x NK603. Using Southern Blots the molecular features of the stacked event are analysed in comparison to the single events 1507 and NK603.

The results confirm the intactness of the gross insert structures in GM maize 1507 x NK603 compared with the modifications in the parental GM events. The methods employed however cannot detect subtle variations in the insert or flanking sequences and most of the data are not conclusive with regard to the copy numbers of inserts, which is in contradiction to the claims of the applicants.

With regard to **compartmental locations** of the inserts and the **organisation of the inserted genetic material** at the insertion sites including sequence data of inserts and flanking sequences the applicant points to information that had been supplied for the parental GM events in previous notifications. According to the results of the Southern Blot analyses, the equivalence to the inserts from the parental GM events is expected. The uncertainties reported for GM maize 1507 regarding the possibility of DNA deletions having occurred during the transformation process (EFSA 2006b) were not considered further.

Concerning the **expression** of the transgenes, a field study at 5 sites in Europe (Spain, France, Bulgaria) had been carried out in 2003. Expression of CRY1F, PAT and CP4 EPSPS was analysed by ELISA in forage and grain of GM maize 1507 x NK603 in a randomized complete block design, with stacked maize and a genetically comparable non GM line as control for a single growing season. The expression levels were similar regardless of herbicide treatment and described as comparable to levels in the parental GM events. Direct comparison of results from field testing between GM maize 1507 x NK603 and the parental GM events was not possible according to this design.

Regarding the expression of **potential fusion proteins** the applicants conclude that on the basis of the above mentioned molecular characterisation the expression of such fusion proteins is not to be expected.

Information on reproduction, dissemination and survivability

The agronomic characteristics of GM maize 1507 x NK603 were evaluated in a field study at 5 sites in Europe in 2003 in comparison to a genetically comparable non GM line. No differences in stalk lodging, root lodging, plant height, stay green (visual estimate of plant health at stage R6), disease incidence and insect damage were detected. Concerning the reproduction parameters (seed germination, seed vigour, pollen shape/colour, time to 50% silking and 50%

pollen shed, ear height) significant differences across locations were found for the latter 3 parameters, but considered to be of no biological significance.

Genetic stability

Here the dossier refers generally to those chapters dealing with the molecular characterisation of the inserts and to the characterisation of transgene expression, as well as to the results of the agronomic evaluation.
Genetic stability is inferred from these analyses without specific testing for more than one generation.

Ability for gene transfer to other organisms

Horizontal gene transfer to bacteria is considered a negligible concern according to the conclusion by the applicants. Plant to plant transfer is restricted to cultivated maize lines in Europe and therefore gene transfer by vertical transmission is considered a negligible risk.

Mechanisms for interaction between GM Plant and target organisms

A general description of the mechanism of the CRY1F protein mode of action in susceptible insects (European Corn Borer, Sesamia spp.) is given. Any potential interactions between transgenes in GM maize 1507 x NK603 were not considered specifically.
With regard to target organisms the effects are claimed to be highly specific on certain target insect pests. However this is drawn from data submitted for the parental GM events.

The potential development of resistance is identified as a risk and considered manageable when applying the insect resistance management plan (IRM plan) in the context of the environmental monitoring plan.

Persistence and invasiveness of GM maize 1507 x NK603, selective advantage or disadvantage

No potential for weediness, persistence and invasiveness is considered with maize as cultivar.
Likewise it is reasoned that the new proteins do not confer any selective advantage to the plants in the natural environment; insect attack is only one of multiple factors that prevent growth of maize outside heavily managed agricultural environments, therefore the CRY1F protein cannot be considered a selective advantage; application of broad spectrum herbicides does not commonly occur in natural environments and therefore PAT/EPSPS proteins do not confer a selective advantage.

Potential effects on non target organisms, biogeochemical processes

The absence of toxicity to non-target organisms of the proteins CRY1F, PAT and CP4 EPSPS is stated in reference to toxicological and allergenicity assessments. No further consideration of the specific nature of a stacked event is indicated, nor have any specific data been submitted for GM maize 1507 x NK603 to back this conclusion.

No effects on biogeochemical processes are considered on the basis of results for the parental GM events used to construct GM maize 1507 x NK603. Conclusions are based on the assessment of effects on soil dwelling organisms, such as earthworms and collembola (EFSA 2005).

Specific effects on specific cultivation, management and harvesting

The effects on specific cultivation, management and harvesting are described as comparable to those of other commercially available maize lines, with the exception of the specific herbicide regime possible for GM maize 1507 x NK603. Regarding this issue the applicants refer to the positive results of British Farm Scale Evaluations for T25 maize (which is tolerant to only one herbicide) and to the proposed environmental monitoring plan.

Due to the ubiquitous occurrence of the CRY1F, PAT and CP4 EPSPS proteins in the soil environment, the applicants propose that no effect on the abiotic environment is to be expected. No specific reference is made to potential combined effects.

In the conclusions from the environmental risk assessment (ERA) according to Dir. 2001/18/EC (Annexes II, III, IV,VII) no adverse effects on human health, animal health or the environment are identified.
The ERA does not make reference to more specific experimental results concerning the environmental behaviour of GM maize 1507 x NK603 than those summarized above.
The only reference made to specific results for the stacked event GM plant with regard to the results of the agronomic evaluation of GM maize 1507 x NK603 concerning issues such as the spread of the GMO in the environment and phenotypic and genetic instability.

Summary with respect to the environmental risk assessment

No specific data for GM maize 1507 x NK603 concerning the assessment of environmental effects are included in the dossier except for the gross molecular characterisation of the inserts with the Southern Blot method in comparison to similar data on the parental GM events, an analysis of the expression of transgenic gene products and an evaluation of agronomic properties in an European field study (5 sites for one growing season). Other conclusions concerning the ERA are drawn from data on the parental GM events used for the construction of GM maize 1507 x NK603.

The following chapters (5.2.2.10 – 5.2.2.13) are based on the application for GM maize 1507 x NK603 according to Reg. (EC) No. 1829/2003 intended for import and processing, and food and feed use (EFSA-GMO-UK-2004-05).

Comparative compositional assessment

The applicant concludes that maize 1507 x NK603 is equivalent to non-GM maize on the basis of a comparative compositional analysis of plant compounds along with agronomic properties.

Field trials were conducted at six locations (four replicates) in major maize growing regions in Chile in 2002-2003. Plots of 1507 x NK603 received either

simultaneous or sequential herbicide treatment. A non-GM control was used as a comparator.

Grain samples were analysed for 53 different parameters including proximates (crude protein, crude fat, crude fiber, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, carbohydrates), fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acids), amino acids (methionine, cysteine, lysine, tryptophan, threonine, isoleucine, histidine, valine, leucine, arginine, phenylalanine, glycine, alanine, aspartic acid, glutamic acid, proline, serine, and tyrosine), minerals (phosphorus, calcium, copper, iron, magnesium, manganese, potassium, sodium, zinc) vitamins (beta-carotene, vitamin B1, vitamin B2, folic acid, and vitamin E), secondary metabolites (inositol, raffinose, furfural, p-coumaric acid, and ferulic acid), and anti-nutrients (phytic acid and trypsin inhibitor).

Statistically significant differences were found across locations for palmitic acid, manganese, potassium, zinc, vitamins B1 and E (grain; glyphosate treatment), crude fat, palmitic acid, stearic acid, oleic acid, linoleic acid, methionine, cystein, magnesium, manganese, potassium, zinc, vitamin B1 and E, p-coumaric and ferulic acid (grain, glufosinate), palmitic acid, stearic acid, methionine, cysteine, aspartic acid, manganese, potassium, zinc, vitamins B1, E and folic acid (grain, glyphosate followed by glufosinate).

Differences detected across locations could not be consistently detected in location based comparisons (confirmed for a maximum of four out of six locations). All mean values were shown to be within literature ranges. For an overview of the statistically significant differences found see Table 6 in Annex 3.

The agronomic traits investigated included early plant population counts, silking, pollen shed, plant height, ear height, stalk lodging, root lodging, final population, stay green, disease incidence, insect damage, pollen shape, pollen colour. Pollen viability has been correlated with pollen shape and colour.
No statistically significant differences were detected.

Toxicity assessment of the whole GM food / feed

No whole food toxicity study was conducted. The applicant considered the comparative analysis of a broiler study with 1507 x NK603 and the safety of the proteins assessed in the course of the authorisation procedures of parental GM events sufficient.

Allergenicity assessment of the whole GM plant

The applicant does not expect that the overall allergenicity of the maize 1507 x NK603 will be changed. No specific concerns were identified because maize has been classified a "less common allergenic food" (Metcalfe 1997)and maize allergies have mainly been caused by pollen.

Nutritional assessment of GM food / feed

Nutritional equivalence is based on compositional equivalence, a 42-day broiler study on maize 1507 x NK603 (no statistically significant differences observed) and a calculation of the theoretical maximum daily intake (TMDI) for acute dietary consumption of the novel proteins. For feed, reference is provided to

compositional analysis data, which were not included and discussed in the Technical Dossier.

For calculating the TMDI the average consumption of maize was used as a basis. The results showed that the TMDI would amount to 20 and 100 µg/person/day (depending on the protein). This is contrasted by toxicity tests where no effect could be detected at high doses (acute toxicity tests conducted in the course of the assessment of parental GM events usually apply 4000 to 5000 mg/kg body weight[1]). From this, a wide margin of safety is concluded.

Comments on risk assessment of GM maize 1507 x NK603

Opinion of the EFSA GMO Panel on GM maize 1507 x NK603

The Scientific Panel on Genetically Modified Organisms delivered an opinion on a preceding application for the placing on the market of genetically modified maize 1507 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 (EFSA 2006a).
The sections on molecular characterisation and the evaluation of transgene expression in this application are structured in a similar manner to the information described above. Therefore the conclusions contained in the Panel's opinion give an indication of how the GMO Panel assesses such information.
The evaluation of environmental risks however is restricted to the scope of the application which does not cover cultivation. Therefore the environmental risk assessment is directed at the potential effects of GM maize 1507 x NK603 according to its intended use for import, processing and uses as food or feed.

The Panel based its assessment on the EFSA GMO Panel guidance document (EFSA, 2004b) and stated that for stacked events derived from interbreeding of existing approved GM lines, the need for further analyses will depend, on a case-by-case basis, on the nature of the genetic modifications.

Compositional Analysis
The Panel confirmed the applicant's conclusion that maize 1507 x NK603 is considered equivalent to conventional maize. Statistically significant differences that occur across locations and on four of six locations (e.g. vitamin E, zinc) of the field trials are not considered to be representing "consistently occurring" differences.

Similarly, agronomic equivalence was confirmed by the Panel.

In a parallel application procedure for maize 1507 x NK603 the scope of which was extended to include cultivation, the national CA that is conducting the ERA asked for the data obtained from previous field trials conducted in Spain.

Toxicity Assessment of whole food/feed
The Panel did not request a 90-day whole–food toxicity study. Evidence stated for justification includes (i) the absence of genetic instability the two events reflected in the expression analysis and the molecular characterisation, (ii) no indication for interaction between the newly expressed proteins (data not shown

[1] Numbers based on experience of the author of this study; not mentioned in the dossier.

in the dossiers), (iii) compositional equivalence, (iv) results from the feeding study with broilers.

Allergenicity assessment of whole plants

In accordance with the conclusion of the applicant, the Panel dismissed the allergenicity risk of the stacked event because maize is not considered to be common allergic food. Food allergies and occupational allergies are of low frequency. Food allergies occur in populations of specific geographic areas. Therefore, a possible increase in endogenous protein expression is unlikely to alter the overall allergenicity of the whole plant or the allergenicity risk for consumers.

Nutritional assessment of GM food/feed

Based especially on the 42-day broiler study and nutritional equivalence between the parental GM events and their respective comparators, the Panel considered maize 1507 x NK603 to be nutritionally equivalent to conventional maize.

By way of conclusion, the GMO panel considers it unlikely that GM maize 1507 x NK603 has any adverse effects on human and animal health or the environment in the context of its intended uses.

However, during the assessment the following requests for information were made to the applicants:

1) Request for additional data on the molecular characterisation of inserts; specifically to clarify ambiguous results in specific Southern Blot experiments. Submitted results did not corroborate the claim of molecular equivalence to single event 1507.
2) Request for data on compositional analyses and environmental evaluations from field trials in 2004 and 2005. EFSA (or more specifically the GMO Panel) considers these data, which would complement data from 2003 that are included in the application, useful with respect to an assessment of the impact on the environment.
3) Questions regarding the level of information on the molecular characterisation of both parental GM events. Additionally, detailed information on the inserted traits in the maize genome for the stacked event GM maize 1507 x NK603 (or at least for both parental GM events) for an assessment of possible recombinations.
4) Questions regarding specific information on the potential adverse effects of GM maize 1507 x NK603 on non-target organisms concerning direct effects due to the toxic effects of CRY1F toxin on non-target arthropods and indirect effects due to changes in the species composition and biomass of weeds with indirect impacts on non-target species (including higher trophic levels).
5) Questions regarding the preferability of more data on compositional analysis and the expression of the insert in addition to those included (data for a single growing season only).
6) Questions regarding consideration of the application of the herbicide Glufosinate ammonium and its direct and indirect effects on the environment both alone and in combination with the *cry1F* and *cp4 epsps* traits.
7) Questions regarding the effects of the continued use of the herbicide Glyphosate on weeds and the potential for shifts in the weed species composition associated with the herbicide regimes.

8) Questions regarding more information on the effects of the relevant herbicides to support justification of the proposed monitoring plan (General surveillance versus Case-specific study components)
9) The Competent Authority concerned with ERA criticised the monitoring plan in general and demanded more specific information on available monitoring data from networks already established in different countries.

Member States Comments on the Notification for GM maize 1507 x NK603

With regard to the application of GM maize 1507 x NK603 for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 (EFSA-GMO-UK-2004-05) a summary of Member States' comments and responses of the EFSA GMO Panel was produced. This document elaborates on the reasoning behind the conclusions in the GMO Panel opinion and sheds light on the different approaches to risk assessment for "stacked events".

With regard to GM maize 1507 x NK603 Member States question the validity, verifiability and interpretation of the compositional analysis.
The Member States' comments clearly point out that the submitted experimental results based on Southern Blots do not provide sufficient information either on molecular equivalence or copy number, in contrary to the conclusions by the applicant. According to EFSA, additional analyses have to be carried out to confirm the results and to address the questions of the structure of the 1507 insert in GM maize 1507 x NK603.

Another concern was the limited data on transgene expression levels. First of all, data from one single growing season (for application EFSA-GMO-UK-2004-05 from sites in Chile with similar climate conditions) were submitted. Second, data concerning expression in parental GM events was not submitted. This caused concern about the presented data giving only a crude estimate of expression levels, making comparison difficult. In conclusion the data thus are regarded as insufficient. In its response, EFSA pointed to the draft guidance on stacked events (EFSA 2006a) and to a weight of evidence approach in this specific case, concluding that within comparable ranges the differences in expression between transgenes in GM maize 1507 x NK603 and the parental GM events are not significant for a safety assessment (EFSA 2006a).

Concern was also raised about limitations on the data used to establish phenotypic and ecologic equivalence, and specifically about limitations of the agronomic evaluation based on one single growing season and a few locations only.

Environmental risk assessment
The comments concerning environmental risk assessment show that, although the scope of the application does not include cultivation, there are different approaches to an assessment. Despite the limited scope, some Member States requested more data for an environmental risk assessment, but the EFSA GMO Panel did not consider additional data necessary for a conclusive risk assessment.
Specifically, a lack of considering potential interactions between traits and their effect on target organisms was noted. To assess these effects, whole-plant

ecotoxicity studies were proposed in order to investigate effects on target organisms and non-target organisms.

Compositional analysis
Member states criticised the insufficient description of the non-GM comparator, the lack of non-herbicide treated 1507 x NK603 control plants and of parental GM events in the comparative compositional assessment. Field trials conducted for one season and at three locations only are considered insufficient. Recent data obtained from field trials in France and Spain should be included in the dossier. The variability in agronomic data between the sites was not analysed.

The Panel did not respond to these requests directly. Instead, rather it reiterated its opinion previously delivered, namely that the controls used were considered adequate by the Panel.

Toxicity assessment
Given that the broiler study is not designed to reveal adverse effects, a 90-day whole-food toxicity study on rats was requested along with additional chronic studies on ruminants and swine exposed to maize 1507 x N603. The Panel responded by reiterating the points made in its previous opinion (see Section 5.2.3.1).

Allergenicity assessment of the whole plant
Member States' comments emphasised the risk of an elevated allergenic potential of the whole GM plant as a possible unintended consequence of the genetic modification. The Panel responded by reiterating the points made in its previous opinion (see Section 5.2.3.1).

Maize MON863 x MON810

General aspects

Applications for authorisation of GM maize MON863 x MON810 were submitted by Monsanto according to Directive 2001/18/EC in 2002 and subsequently under Regulation (EC) No 1829/2003 in 2005.
The scope of the applications includes import, processing and feed use of GM maize MON863 x MON810 and the use of GM maize MON863 x MON810 in food and feeds.

GM maize MON863 x MON810 was developed by combining single maize events MON863 harbouring *cry3Bb1* and *nptII* genes and MON810 harbouring the *cry1Ab* transgene. The resulting "stacked event" GM Plant therefore exhibits resistance to lepidopteran and coleopteran insect pests due to the inserted CRY-Toxins.

The parental GM events MON863 and MON810 have been the subject of earlier assessments. For MON863, the EFSA opinion was in favour of its authorisation. MON810 was approved under Directive 90/220/EEC by Commission Decision 98/294/EC. The use of food and food ingredients from MON 810 maize was notified in 1997 under Article 5 of Regulation (EC) No 258/97.
The GMO Panel considers it unlikely that GM maize MON863 x MON810 has any adverse effects on human and animal health or the environment in the context of its proposed use. A number of Member States have commented on the

applications and expressed concerns with regard to specific issues of the application.

Risk assessment according to the applicant

No new modification was introduced into GM maize MON863 x MON810. Accordingly, the applicant describes most of the traits and characteristics of the "stacked event" as being the same as those of the parental GM events used in the production of GM maize MON863 x MON810.

Molecular Characterisation

The applicant states in the dossier that the genome of GM maize MON863 x MON810 contains two different inserts derived from the parental GM events. Specific analyses have been conducted concerning the preservation of the molecular structure of these inserts. Using Southern Blots, the molecular features of the "stacked event", such as the copy number as well as the gross structure and organisation of the inserts were analysed in comparison to the parental GM events MON863 and MON810.

The results confirm the intactness of the gross insert structures in GM maize MON863 x MON810 compared with the modifications in the parental GM events. The methods employed however cannot detect subtle variations in the insert or flanking sequences. Additional copies of the inserts producing comparable fragments upon enzymatic digestion could not be detected on the basis of the results supplied in the dossier.
The fingerprint analysis included in the application for GM maize MON863 x MON810 according to the Reg. (EC) No. 1829/2003 (EFSA/GMO/DE/2004/03) which is based upon Southern Blots with two different digestions reduces the possibility of other inserts not being detected.
Since data do not provide quantitative evidence of concentrations of the analysed sequence elements, it is not possible to assess the relevance of different intensities of signals in Southern Blots without additional data.

With regard to **compartmental locations** of the inserts, the information included relates to the characterisation of the inserts as a single complete copy located in the plant nuclear genome and concludes that the location of the inserts in GM maize MON863 x MON810 is expected to be the same, based upon the construction of the "stacked event" by crossing.

For detailed information on the organisation of inserts and flanking sequences in GM maize MON863 x MON810 the applicant refers to information on the parental GM events. No specific examination of the detailed molecular structure of the inserts in the "stacked event" is included.

Concerning the **expression of the transgenes,** a field study at four sites in Argentina for a single growing season was carried out. The expression of transgenes was analysed by ELISA in forage and grain of GM maize MON863 x MON810 and additionally in other maize tissues to assess exposure of non-target species (young leaves, root and pollen for CRY3Bb1 and young leaves and pollen for CRY1Ab). The data were analysed in comparison to the respective parental GM event and a non-GM maize line of comparable genetic make-up.

The dossiers show that the average levels of CRY3Bb1 and CRY1Ab protein measured in most of the samples were higher in GM maize MON863 x MON810 than the respective levels in parental GM events, whereas NPTII levels are comparable between GM maize MON863 x MON810 and the parental GM events lines. It is also shown that there is a notable difference in expression levels of CRY3Bb1 in MON863 between trials in Argentina and trials conducted in the USA during the 1999 growing season. The differences are not concluded to be a safety concern.
According to the applicant the results indicate that the range of expression of the transgenes (*cry3Bb1, cry1Ab and nptII*) in the GM maize MON863 x MON810 is comparable to levels in the respective parental GM events.

Regarding the expression of **potential fusion proteins,** the applicants conclude that on the basis of the molecular characterisation of the inserts, an expression of such fusion proteins is not to be expected.

Information on reproduction, dissemination and survivability

Referring to the conclusions drawn for the parental GM events the applicant does not anticipate changes in the reproductive capabilities of GM maize MON863 x MON810. According to this, similar behaviour with conventional maize lines is expected.
The application for GM maize MON863 x MON810 according to Reg. (EC) No. 1829/2003 (EFSA/GMO/DE/2004/03) however includes more information on the properties in question:
The applicant refers to comparative assessments of phenotypic and agronomic characteristics of the parental GM events in comparison to conventional maize varieties at multiple sites. However these claims are not referenced in the dossier.
Additionally, observational data from field tests with GM maize MON863 x MON810 in the USA are addressed to conclude that GM maize MON863 x MON810 does not exhibit significant changes in its dispersal and survival characteristics. The parameter set which supports this conclusion includes growth habit, ear drop and morphological and developmental characteristics including seedling vigour, ear and plant height, stalk or root lodging, and yield.
Furthermore the dossier refers to the data from the compositional analysis to support the overall conclusion that agronomic equivalence of GM maize MON863 x MON810 can be expected. However no specific data are referenced in the dossier.

Genetic stability

The dossiers generally refer to the molecular characterisation of inserts and the expression of the traits to support the notion that GM maize MON863 x MON810 is genetically stable. Results of the assessment of parental GM events do not indicate any intrinsic instability features associated with the respective maize lines.
Apart from a general description of sources of genetic instability in maize reproduction and the relevance for the transgenic traits in GM maize MON863 x MON810 no specific data to support the conclusions are included.

Ability for gene transfer to other organisms

Horizontal gene transfer to bacteria is considered to be of no specific concern because the inserts in GM maize MON863 x MON810 do not constitute genetic transfer functions. Plant to plant transfer is considered to be of no concern for applications that do not include cultivation in their scope.

Persistence and Invasiveness of GM maize MON863 x MON810, Selective advantage or disadvantage

Based on the general characteristics of maize as a crop in European agricultural environments it is concluded that the likelihood of GM maize MON863 x MON810 to exhibit increased persistence or invasiveness is negligible. This conclusion is drawn with regard to agronomic field test data on the parental GM events that demonstrate that the introduced traits have no influence on reproductive features and therefore no changes in persistence and invasiveness are to be expected.

In consideration of the poor fitness of the offspring of hybrid maize lines like MON863 x MON810 no selective advantage is expected due to the introduced traits. The dossier suggests that only under conditions of high infestation of coleopteran and lepidopteran pest species GM maize MON863 x MON810 would have selective advantages compared to other maize varieties.

Potential for gene transfer

The conclusion that there are no risks associated with GM maize MON863 x MON810 concerning gene transfer is based upon the assumption that no potential for gene transfer to wild relatives of maize is expected in European environments and that the likelihood of a transfer to other maize crops and its consequences is expected to be limited.

Interactions between GM maize MON863 x MON810 and target organisms

The considerations of environmental impacts of the "stacked event" are restricted to the potential for development of resistance in the target species to the CRY3Bb1 and CRY1Ab toxins. According to the applicant, this development would depend on extensive and repeated cultivation of GM maize MON863 x MON810, exerting a high selective pressure which would favour the development and spread of resistance in insect populations.
This is considered to be unlikely under conditions of insect resistance management plans that would have to be followed when cultivating GM maize MON863 x MON810 in Europe.
Potential interactions between transgenes in GM maize MON863 x MON810 were not considered specifically.

Potential effects on non-target organisms and biogeochemical processes

This issue is claimed to be irrelevant with the proposed scope of the application. Therefore no data are presented to evaluate any potential effect on non-target organisms by GM maize MON863 x MON810.

No effects on biogeochemical processes are expected according to the applicant for reasons of the rapid degradation of CRY proteins in the soil.

The effects on specific cultivation, management and harvesting are described as comparable to those of other commercially available maize lines, with the exception of changes in pest management due to replacement of chemical insecticides by GM maize MON863 x MON810.

Concluding from the environmental risk assessment according to Dir. 2001/18/EC (Annexes II, III, IV, VII), no adverse effects to human health, animal health or the environment have been identified.

Summary with respect to environmental risk assessment

No specific data for GM maize MON863 x MON810 concerning the assessment of environmental effects were documented in the dossier beyond the gross molecular characterisation of the inserts with the Southern Blot method (in comparison to similar data on the parental GM events), the analysis of the expression of transgenic gene products in a field study in Argentina (four sites for one growing season). Other conclusions concerning the ERA are drawn from data on the parental GM events used for the construction of GM maize MON863 x MON810.

Comparative compositional assessment

Compositional analysis was conducted on forage and grain from MON863 x MON810 samples obtained from field trails in Argentina (replicated field trials, four sites, one season). A non-GM maize line with a similar genetic background, the parental GM events, and four commercial hybrids were used as control.

Grain samples were analysed to measure proximates (protein, fat, ash, carbohydrate, moisture), acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamin B1, vitamin B2, vitamin E, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), folic acid, phytic acid, trypsin inhibitor, ferulic acid, inositol, raffinose, 2-furaldehyde (furfural) and p-coumaric acid content of grain; forage samples were analysed for proximates, ADF and NDF.

Across the sites, 12 statistically significant differences of 58 comparisons were found (see Table 6 in Annex 3). More differences were detected in site by site analysis. For all 71 significant differences ($p<0,05$) out of a total of 290 comparisons, the range of the values for MON863 x MON810 was within the 99% tolerance interval or the range of values of the commercial hybrid.

For agronomic equivalence the dossier referred to observations of phenotypic characteristics (ear drop, growth habit) and morphological and developmental characteristics (seedling, vigour, ear and plant height, stalk or root lodging and yield). According to the applicant these observations would support the claim of agronomic equivalence. However, the dossier does not provide any reference to more detailed data.

The applicant concludes that in terms of substantial and nutritional equivalence as well as safety of the introduced proteins, no difference is expected from the production of MON863 x MON810 compared to traditional maize.

Toxicity Assessment of the whole GM food / feed

Regarding safety, the conclusion of the applicant is based on the studies and evidence listed below:
First, pleiotropic effects are deemed unlikely because of the demonstration of substantial equivalence based on compositional analysis and comparative agronomic and phenotypic assessments. Second, the safety of the introduced proteins has already been demonstrated in the assessment of the parental GM events. Third, no toxicity relevant interaction of proteins is expected for a number of reasons:

- Different modes of action of CRY3Bb1, CRY1Ab and NPTII proteins: CRY1Ab is considered to be specific to lepidopteran insects, CRY3Bb1 to coleopteran insects. No evidence is available on Cry1-associated toxicity to coleopteran, nor on CRY3-associated toxicity to lepidopteran species. Furthermore, no such additive and synergistic effects have been detected in field testing since 1999. Modes and sites of biological activity are also different in the case of NPTII.

- Expression in very low quantities in MON863 x MON810: "significant interactions" between the two proteins are considered unlikely given the low levels of all three proteins in the plant (as revealed by expression analysis of the transgenes in MON863 x MON810)

- Evidence from the mammalian toxicity assessment literature of chemicals: interactions do not occur in mixtures of chemicals if substances are administered below the No Observed Adverse Effect Level (NOAEL) – which is the case for both the parental GM events MON863 and MON810

- Broiler studies on MON863 x MON810 do not indicate adverse health effects

- History of the safe use of mixtures of MON863 and MON810 grains and of the individual proteins (no further information provided)

Allergenicity Assessment of the whole GM plant

Maize is not considered a "common allergenic food" as food allergies are of low frequency and mainly occur in populations of specific geographic areas. Occupational allergies are also considered to be rare. MON863 x MON810 is not assumed to alter food consumption, and thus, over-expression of any endogenous protein is not expected to alter the overall allergenicity of the plant for consumers.

Nutritional Assessment of GM food / feed

In the case of food, the introduced traits are not expected by the applicant to alter the nutritional properties of the plants for a number of reasons. First, all traits aim at agronomic properties. Second, substantial equivalence of MON863 x

MON810 has been demonstrated. Third, broiler studies comparing grain from MON863 x MON810 to a non-transgenic control hybrid or commercially available reference hybrid confirm nutritional equivalence. Thus, according to the notifier no change of diet or intake or any subsequent nutritional impacts can be expected.

In the case of feed, nutritional equivalence of input traits is assumed on the basis of substantial equivalence (see Clark and Ipharraguerre 2001 and Flachowky and Aulrich 2001)[2]. Furthermore, the broiler study is considered to provide confirmatory evidence.

Comments on the risk assessment of GM maize MON863 x MON810

Scientific opinion on applications for GM maize MON863 x MON810

According to the application for GM maize MON863 x MON810 under Dir. 2001/18/EC, an assessment report was prepared by the German authority assessing the application.
Second, the Scientific Panel on Genetically Modified Organisms delivered an opinion on the application for the placing on the market of GM maize MON863 x MON810, for food and feed use, and import and processing under Regulation (EC) No 1829/2003 (EFSA 2005).
In both opinions, the evaluation of environmental risks is restricted to the scope of the application which does not cover cultivation. Therefore the environmental risk assessment is directed at the potential effects of GM maize MON863 x MON810 according to the intended use for import, processing and use as food or feed.

In summary, it was concluded that no adverse effects were to be expected. Concerning the *nptII*-antibiotic resistance gene present in MON863 x MON810, the report refers to conditions by the European Commission for the phasing out of antibiotic resistance markers. The following issues indicate how certain conclusions were achieved:

- The assessment report according to Dir. 2001/18/EC accepts the molecular characterisation of the parental GM events which were combined to construct GM maize MON863 x MON810. No reference is made to the molecular characterisation of GM maize MON863 x MON810 itself.
- Expression analyses are documented in comparison to data for the parental GM events. Apparent differences in expression levels between trials in USA and Argentina are not discussed.
- Regarding the agronomic characteristics, the conclusions for MON863 x MON810 were based on results of the assessment of parental GM events.
- The assessment of toxicological and allergenic properties was based upon evaluations of transgenic proteins. The assessment did not specifically include potential interactions of the inserted traits.
- Likewise, results from feeding studies employing MON863 and MON810 are applied for the safety assessment of GM maize MON863 x MON810.
- Compositional analysis data on GM maize MON863 x MON810 in comparison to an isogenic comparator was accepted and substantial equivalence except for the transgenic proteins was accepted.

[2] References taken from the Technical Dossier (Monsanto 2004).

- The ERA conclusions were accepted with regard to the scope of the application and the proposed monitoring plan was accepted providing minor changes were made.

The assessment by EFSA was finalised according to Regulation (EC) No. 1829/2003, after EFSA had made a request for an additional 90-day rat study on GM maize MON863 x MON810 and the applicant had submitted the study.

- Concerning molecular characterisation, the Panel agrees with the conclusions reached by the applicant, namely that insert structures are retained and their stability is demonstrated.
- It is accepted that the data from expression analyses do not indicate safety concerns. That the "stacked event" produces higher levels of transgenic CRY proteins was noted but not considered a safety concern.
- As regards agronomic evaluation, the panel accepts the conclusions given in the dossier and accepts the absence of further data.
- Regarding agronomic characteristics the assessment of parental GM events was accepted as a basis for claims regarding MON863 x MON810.
- The environmental risk assessment was prepared with regard to the scope of the application and agrees that any unintended environmental effects of GM maize MON863 x MON810 are no different from other maize varieties.

Compositional Analysis

Of all 71 significant differences found in the compositional analysis only linolenic acid was mentioned. The average level of linolenic acid in kernels of MON 863 x MON 810 maize was significantly decreased compared with that of all comparators in each separate location. The Panel regards this difference as "being small and within the range found in the literature and within the natural variation". The Panel also referred to reports that the fatty acid composition of maize kernels can vary substantially between maize varieties, and is influenced particularly by genetic factors (Dunlap et al., 1995). The Panel concluded that the difference in linolenic acid is not meaningful from a biological point of view.

The Panel did not anticipate interactions which would alter the agronomic characteristics. Therefore, the Panel accepted the absence of agronomic data.

Toxicity Assessment of whole plants

The Panel acknowledged that 90-day subchronic toxicity studies of the parental GM events did not reveal any adverse effects. Neither did the Panel anticipate any pleiotropic effects resulting from the mode of action of the proteins. Nevertheless, the Panel requested an additional 90-day study with MON 863 x MON 810. The Panel was apparently divided over the necessity for such a study and EFSA therefore decided to require the study from the applicant. However, EFSA highlights the confirmatory status of the study and did not include it in its formal completeness check (EFSA 2004a).

The results showed small deviations in food consumption by female rats. Statistically significant differences observed with mean corpuscular haemoglobin concentrations in male animals were considered not dose-related and not accompanied by changes in the red blood cell counts, haematocrit values, and other red blood cell parameters. Another statistically significant, but slight difference in basophil counts was observed but only in males that received the 11% test diet. The Panel considers the changes observed to be of no toxicological relevance. Statistically significant differences observed with organ

weights, e.g. lower mean absolute and relative thyroid/parathyroid weights were found not to show a dose-response relationship and microscopic observations failed to reveal any abnormalities.

Member States Comments on the Notification for GM maize MON863 x MON810

Of the application for GM maize MON863 x MON810 for food and feed use, and import and processing under Regulation (EC) No 1829/2003 (EFSA-GMO-DE-2004-03) a summary of Member States' comments and responses of the EFSA GMO Panel was published on the EFSA website. This document elaborates on the reasoning behind the conclusions contained in the GMO Panel opinion and sheds light on different approaches to risk assessment for "stacked events".

The Member States' comments clearly point out that the molecular characterisation scheme by Southern Blots only indicates conservation of the gross structure of the insert and should be complemented by methods allowing for an analysis of subtle rearrangements.

Concern was expressed about transgene expression evaluation. First of all, data from a single growing season in Argentina were submitted. Second, expression of GM maize CRY proteins is higher in MON863 x MON810 than CRY expression in parental GM events. Furthermore remarkable differences in expression levels between field trials in Argentina and the USA were noted, but were not addressed appropriately.

Concern was also raised about limitations on the data used to establish phenotypic and ecologic equivalence, and specifically about limitations on the agronomic evaluation considered insufficient for an assessment of any potential changes in ecological characteristics.
Furthermore a large number of significant statistical differences in the compositional analysis were noted, which raised concern whether substantial equivalence could be confirmed by the data.

The comments concerning environmental risk assessment show that although the scope of the application does not include cultivation, there are different approaches. Despite the limited scope, some Member States requested additional data on the "stacked event" itself.

Compositional analysis

Member States questioned the validity, verifiability and interpretation of the compositional analysis. First, agronomic equivalence of MON863 x MON810 is not backed up by appropriate studies. The studies on MON863 did not use the entire data set of the US field trials; they were restricted to one year and four locations. Between-site variability was not analysed and the parameters observed allowed no assessment of a potential change of ecological characteristics. EFSA did not directly address this point of criticism, but argued on the basis of its draft guidance document that in this case (both parental lines had been sufficiently assessed before) one year field trialling of events combined by crossing is acceptable. Consequently, the Argentinian field trials were considered sufficient and the Panel was satisfied with the data received. Generally, the Panel did not anticipate any interaction that would alter the agronomic characteristics (without specifying whether this view had been an assumption of the Panel from the start

or was reached as a consequence, and without specifying on what in particular grounds the assumption was made).

Second, the fact that the large number of statistical differences between MON863 x MON810 and the control lines (total comparisons 290; 71 significant differences with MON846; 59 significant differences with MON863; 122 significant differences with MON810; 142 significant differences with commercial lines) plus the higher expression levels in MON863 x MON810 compared to the parental GM events (see above) can be interpreted as substantial equivalence to conventional maize varieties is questioned.

In its reply to these concerns, the EFSA Panel argued that all differences were within normal ranges of variation and that its interpretation is backed up further by the results of the compositional analysis of the parental GM events.

Toxicity assessment of whole food / feed
On the basis of the large number of statistically significant differences in compositional analysis, a 90-day whole-food toxicity study was requested. Given the similarities in the mode of action of both CRY toxins ("gut toxins, receptor bound molecules induce pore formation and disintegration of the gut tissue"), additional chronic studies should be conducted on swine and ruminants, which will be exposed to MON863 x MON810 feed after commercialisation. This is in contradiction to both the applicant's and the Panel's views.

The Panel referred to the fact that it had requested such a 90-day study. It did not directly mention the request for additional chronic studies, but implied that the 90-day study did not provide results that would justify further studies.

Allergenicity assessment of the whole plant
Member States' comments also pointed out that no data were provided to assess the potential effects of the genetic modification of the overall allergenicity of the whole plant. EFSA supported the view of the applicant that maize is not considered a common allergenic food and will not pose relevant risks in occupational contexts. Moreover according to EFSA, no validated test is available that would provide a higher guarantee of safety.

Related comments questioned the allergenicity assessments of introduced proteins that were conducted in the course of assessing the parental GM events.

Comparative analysis of case studies

A list of specific data provided by the notifiers for the stacked events 1507 x NK603 and MON863 x MON810 is given in the following Table 3.

Table 3: Overview hybrid-specific safety studies provided for risk assessment of 1507 x NK603 and MON863 x MON810

Study / Data provided	Maize 1507 x NK603[f]	Maize MON863xMON810
Molecular characterisation	**Y** (Southern Blot)	**Y** (Southern Blot)
Expression of inserts	**Y** (1 season, 5 sites; comparator: isogenic non-GM)	**Y** (1 season, 4 sites; comparator: isogenic non-GM, parental GM events)
Genetic stability	N	N

Study / Data provided	Maize 1507 x NK603[f]	Maize MON863xMON810
Agronomic evaluation	**Y** (1 season, 5 sites Europe; comparator: isogenic non-GM)	Y (USA; Specific data not referenced)
Environmental assessment	**N** (ref. to Agronomic evaluation)	**N**
Gene transfer	**N**	**N**
Persistence / Invasiveness	**N**	**N**
Interaction target organisms	**N**	**N**
Interactions non-target organisms	**N**	**N**
Interactions abiotic environment	**N**	**N**
Effects cultivation	**N**	**N**
Toxicity Assessment		
Interaction of proteins	N.a.[d]	N.a.[c]
ORFs	*Cry1F*: 24 ORFs in maize 1507 tested for homology to toxins and allergens	?
Whole-food toxicity study	N	90-day subchronic toxicity study in rats[e]
Allergenicity assessment		
Whole-plant study	Food and occupational allergies occurring only in rare cases.	Food and occupational allergies occurring only in rare cases.
Compositional Analysis		
Parameter	According to OECD	According to OECD
Comparator	"non-GM control with comparable genetic background" treated with glyphosate (i), glufosinate (ii) and glyphosate followed by glufosinate	Non-GM lines, commercial hybrids, parental GM events
Number of sites	6	4 (with replicates)
Number of seasons	1	1
Statistical significant differences	32 (across locations)	Total of 71 in 290 comparisons
Exceeding literature ranges	N	N[a]
Geographical spreading	Chile (3 regions)	Argentina
Agronomic analysis	N[b]	Y
Agronomic evaluation	**Y**	Y[b] (specific data not referenced)
Nutritional equivalence		
Feed conversion studies	Broiler study and dairy cows	Broiler study

N … no study conducted, Y … study conducted

a) Within the 99% tolerance interval of the range of values of the commercial hybrid.

b) Briefly mentioned but no data provided. Unclear if systematically investigated.

c) No interaction anticipated based on different modes of action of the CRY proteins, low expression levels, low likelihood of interaction if substances are administered below NOEL, results of a broiler study, and history of safe use including mixtures of MON810 and MON863 grains.

d) Assumption of absence of interactions not justified in the dossier.

e) Requested by EFSA.

f) For this stacked event two dossiers were investigated. Environmentally specific aspects were taken from the Directive 2001/18/EC Dossier including cultivation. The data on compositional analysis, toxicity, allergenicity, and nutritional assessment were taken from the Regulation (EC) No.1829/2003 Dossier. Both dossiers are similar with respect to toxicity, allergenicity, and nutritional assessment. Compositional analysis of plant compounds, agronomic properties and expression of the transgene was conducted with samples from Argentinian field trials in the case of the Regulation (EC) No.1829/2003 Dossier and from European field trials in the case of the Directive 2001/18/EC Dossier.

By way of summary, the results presented in the previous table indicate that

- only very few conclusions for the safety evaluation of both "stacked events" were based on specific studies with the "stacked events" themselves,
- most of the conclusions regarding safety evaluation were based on corresponding data generated for and conclusions drawn from the assessment of the parental GM events used in the development of the "stacked events",
- only few studies are suitable to indicate potential effects due to interactions of the transgenic traits incorporated in the "stacked events",
- such interactions were not considered specifically for environmental risk assessments in spite of a potential for such effects being relevant for the environmental risk assessment of both applications (combined effects of herbicide application for 1507 x NK603, combined effects of CRY-toxins for MON863 x MON810)

Molecular characterisation

The **molecular characterisation of inserts** is based on Southern Blot results from the stacked events in direct comparison to similar analyses on the parental GM events.
In this way the insert structure can only be analysed in general, since Southern Blots do not generate quantitative but only qualitative results. Furthermore the data only confirm the gross equivalence of the inserts; subtle changes are difficult to detect with the methods employed. These limitations were mentioned as a cause for concern in some Member States' comments. Nevertheless the results of the tests were accepted by EFSA which concluded that molecular equivalence of inserts in the "stacked event" and the parental GM events is established.
For GM maize 1507 x NK603 the presented results were not conclusive with regard to assessing the copy number. This is in contradiction to the respective assumption in the application. In this case additional tests by Southern Blot were necessary to confirm the conclusion that copy numbers of inserts in the stacked event were similar compared to the parental GM events.

In summary the characterisation of inserted traits was conducted with methods that can only roughly establish equivalence with the inserts in the parental GM

events. Evidence was taken from the assessment of parental GM events on the assumption that the breeding methods to construct the stacked events do not necessarily and inherently lead to alterations in the fine structure of the inserts. Other changes in the genome of the "stacked events" were not assessed and not regarded as crucial for an assessment.

Based on the available data for expression of transgenic traits and the agronomic performance, the conclusion was drawn that alterations leading to major changes in the traits did not occur. Again this conclusion is only valid for changes which affect the detectability in ELISA tests and significantly affect the function of the transgenic proteins. Any other changes e.g. changes in the traits which cannot be detected by the methods used or alterations of other genetic elements which interact with the analysed proteins, cannot be detected by the methods currently used, but may still be relevant for risk/safety.

Regarding the **assessment of expression** of the transgenes, both applications limit the specific analyses to a single growing season and to 4-5 different sites. The applications furthermore differ with regard to the comparators used. For 1507 x NK603 a comparison was made to a non-GM maize variety with comparable background, whereas for MON863 x MON810 both an isogenic non-GM variety and the respective parental GM events were used. For MON863 x MON810 a direct comparison with expression in the parental lines can be made. Without data allowing for direct comparison, an assessment can only be made by comparing data from different sites and/or years. Variability of expression between different field tests as encountered with MON863 x MON810 adds undesirable uncertainties to the assessment.
With respect to MON863 x MON810, such differences in expression analyses between geographically distinct test sites were reported but not considered to be relevant as a safety issue.
The limited data on expression analysis caused concern, and lead to a request by EFSA for more data (from field tests at the test sites in consecutive years), thereby contradicting the guidance document in that expression data from a single season are sufficient for assessment in those cases where the parental GM events were assessed according to EFSA general guidelines (EFSA 2004b)

Environmental assessment

Specific data for environmental assessments were generated in the evaluation of the agronomic properties of the stacked events. These data are documented and referenced properly only for 1507 x NK603, the design of the tests being similar to the tests for expression analysis and compositional analysis.
These data are not suitable as indicators of the environmental impact of the "stacked events" and primarily suited to indicate the functionality of the transgenic traits (in case of the analysed applications: herbicide tolerance and insect resistance). They are of limited significance for assessing reproduction and dissemination and not suited to assess survivability.
The significance of these data for any other environmental effects is very limited. Specifically unintended effects due to the combination of traits and genetic backgrounds in the stacked event would not necessarily be detected by an evaluation of germination, seed vigour, plant/ear height, time to silking/pollen shed, stalk/root lodging, population numbers, stay green, disease, insect damage, pollen shape/colour as done for 1507 x NK603.

For all other issues considered in the environmental assessments for both stacked events, the conclusions are drawn in reference to data for the parental GM events. This is ignoring the fact that the interactions of stacked events GMOs with their environment might be altered due to the combined traits. Changes in the expression analyses – as far as those can be detected with the test design utilized - are indicative of such a possibility (De Schrijver 2006).

In case of traits with a potentially synergistic mode of action (e.g. combined effects of different toxins in MON863 x MON810, combined effects of application of two different herbicides with 1507 x NK603) data on the individual parental GM events are not sufficient for an assessment.
Due to the limited scope of the application for MON863 x MON810 (import, processing and feed use according to Dir. 2001/18/EC and for food and feed use according to Reg. (EC) No. 1829/2003 it cannot be deduced how an environmental assessment would be approached in the case of two different Bt-toxins with potential synergistic effects. From a scientific viewpoint, such combination effects need consideration with respect to cross-resistance development and synergistic effects towards target and non-target organisms (De Schrijver 2006).

The monitoring plans were regarded as not sufficient in Member States' comments and their suitability to detect any unintended effects due to the combination of traits and genetic backgrounds seems questionable.

Compositional analysis

The compositional analysis provided in the dossiers does not differ from similar analyses in the case of parental GM events. Compositional analysis is largely conducted in accordance to OECD recommendations and data on agronomic properties are provided at least in the case of maize 1507 x NK603.

Differences in the number of sites, the field trial design, the choice of analytes, and the level of detailed information provided in the dossier and in the critical comments by Member States are similar to those of parental GM events (e.g. Spök et al. 2004, Dolezel et al. 2006).

Some observations can, however, be made that are more specific to "stacked events":

- Field trials are conducted for one season only
- Parental GM events are not consistently included as control for compositional analysis but also for agronomic and expression studies (e.g. not included for compositional and agronomic studies in case of 1507 x NK603).

Both aspects met with criticism from Member States. Field trials conducted for one season only are not considered valid because of climatic variations. The reduced duration of field trials compared to full risk assessments is, however, in line with the EFSA Draft Guidance (EFSA 2006a) and the EuropaBio Guidance (2005). Parental GM events are not required by the EFSA Draft Guidance and are considered an optional comparator by the EuropaBio Guidance. De Schrijver et al. (2006) proposes parental GM events as control for expression studies but it is

not entirely clear whether parental GM events should also be included in trials for compositional and agronomic equivalence.

Toxicity assessment of whole food / feed

In principle, the data provided for the parental GM events on toxicity assessments of introduced proteins and the conclusions for compositional, agronomic and nutritional equivalence based on comparative compositional studies and feed conversion studies were fairly similar in both dossiers and are considered valid as no changes could be detected on the molecular level.

The possibility of interaction of introduced proteins (giving rise to adverse additive or synergistic effects) and the need for whole food toxicity studies are issues specific to "stacked events".

Whole food toxicity studies on rodents were requested by EFSA in the case of MON810 x MON863 and explicitly considered not to be necessary in the case of 1507 x NK603. In the EFSA opinion (EFSA 2005) it is not entirely clear why the toxicity study was requested in the former case. Furthermore, the Panel was apparently divided over the need for such a study. Possible clues can be derived from different characteristics of the events and differences in the dossiers.

First, an interaction of the introduced proteins is considered unlikely by applicants and the EFSA Panel in both cases. However, these conclusions are based on assumptions of the likelihood of such interactions, considering mode of action, expression level, evidence from broiler studies etc. In the case of MON863 x MON810 two CRY proteins (CRY1Ab, CRY3Bp1), in the case of maize 1507 x NK603 two proteins conferring herbicide tolerance to different herbicides and one CRY protein (PAT, CP4 EPSPS, CRY1F) were introduced[3]. Interaction between the two CRY proteins might have been considered less unlikely by the Panel and by the applicant as well. This is reflected in a more extensive reasoning in the MON863 x MON810 dossier. Although the applicant argues that the mode of action of the two CRY proteins is entirely different, different views were voiced from Member States (see Section 5.3.3.2) which considered both cases to have similar modes of action, differing only with regard to the host range. This raises the possibility of adverse additive or synergistic effects.

Second, and perhaps more importantly, a large number of statistically significant differences in compositional analysis and also in the expression studies were described in the MON863 x MON810 dossier and critically commented by Member States (see Section 5.3.3.2). Furthermore, the MON863 x MON810 dossiers also included less comprehensive agronomic data.

Molecular characterisation and genetic stability is another factor likely to play a key role in triggering whole food studies. However, no explicit links have been made by the Panel.

Whole food toxicity studies on rodents are not considered a standard requirement by EFSA for a full risk assessment (EFSA 2004b), although an increasing number of recent dossiers include such studies. As the EFSA opinions

[3] In addition, the marker protein NPTII is present but is not relevant to the discussion here.

do not provide any systematic clues as to what kind of supplementing evidence was requested from the applicant by the Panel and why the Panel arrived at the decision to require this additional information, it remains largely unclear for future cases what triggers such a study.

Whole-food toxicity studies as a case-by-case requirement are also reflected in the Draft Guidance on "stacked events" (EFSA 2006a) while the EuropaBio Guidance does not require whole food toxicity studies (EuropaBio 2005). De Schrijver et al. (2006) proposes altered levels of expression and molecular stability as possible triggers.

Allergenicity assessment of the whole plant

The risk of enhanced allergenic properties of the whole plant is disregarded in both cases by both the applicant and the EFSA Panel. The main reason given is that maize is not considered a common food allergen and that occupational allergies occur in rare cases only.
This line of reasoning has been criticised by Member States and in the scientific literature (Spök et al. 2005). Nevertheless, it is in line with what is currently being done in risk assessments of parental GM events and with the EFSA General Guidance (EFSA 2004b). Guidance documents for "stacked events" do not even mention this issue (EFSA 2006a, EuropaBio 2005). De Schrijver et al. (2006) emphasized the possibility of altered overall allergenicity, but did not provide suggestions for dealing with this problem.
This appears to be a particular weakness of risk assessments that is likely to remain until suitable animal models will be developed. This weakness is relevant for "stacked events". However, it is not specific to the assessment of "stacked events".

Nutritional assessment of GM food / feed

Nutritional assessments are conducted in a fairly similar way to risk assessments of parental GM events and they are mainly based on the demonstration of compositional equivalence, on the safety of introduced proteins and on broiler studies (feed conversion, digestibility).
The latter type of studies is considered a case-specific requirement in both the EFSA Draft Guidance (EFSA 2006a) and the EuropaBio Guidance (EuropaBio 2005). These studies serve several purposes and are therefore included in almost all dossiers on GM crops, food and feed. Applicants refer to them for agronomic properties in the case of feed use, for nutritional properties in cases of food and feed, as additional safety assurance in the case of possible adverse health effects and – in the case of "stacked events" - for absence of interaction of introduced proteins. It is highly questionable, though, whether the design of these feeding studies actually allows for relevant conclusions.

Open questions and possible way forward

On the basis of the analysis provided in this study and taking into account the debate on appropriate risk assessment requirements in the scientific literature, as well as the diverging views of national Competent Authorities as reflected in the Member States' comments on dossiers, a number of issues and questions can be identified that are specific to or of special importance for the risk assessment

of "stacked events". These questions are briefly described in the following. The issues and questions refer to the need for further clarification, more explicit guidance and more explicit reasoning in EFSA opinions and comments.

Choice of appropriate methods for molecular characterisation

An appropriate assessment of the molecular preservation of integrated traits and flanking sequences is a necessary step in the risk assessment of "stacked events". However the currently used methods confirm the gross insert structure only. An adequate strategy to identify minor changes in the inserted traits and flanking elements needs to be pursued in order to assess any alterations with a potential relevance for further risk assessment. Complementing PCR analyses as indicated in the EFSA guidance document (EFSA 2006a) could supplement information gathered by Southern Blots. Information on the chromosomal location of the traits can complement an assessment of the insert copy number and is relevant for an assessment of the recombination potential as indicated in the Member States' comments. In case any differences (at the DNA level or protein level according to the expression analysis) to the respective data from parental GM events are detected, further sequence analysis may be considered necessary.

An open question which may be difficult to address is the assessment of any other genetic changes in the genome of the "stacked event" as a consequence of the construction of the stacked event hybrid or any rearrangements, which are a consequence of initial genetic modifications, but were not detected in the assessment of parental GM events. There is a certain potential for such changes (Latham et al. 2006), but no conclusive scientific evidence of whether such changes could be relevant in terms of risk assessment for "stacked events".

Choice of controls in comparative compositional analysis

The use of parental GM events as additional control in comparative analysis for gene expression studies, compound analysis, and agronomic equivalence has certain merits with respect to direct comparability. First, comparative studies of parental GM events date back to the early 1990s in some cases and might no longer comply with most recent standards (e.g. maize MON810). Second, possible differences in soil, climatic and other conditions make it more difficult to compare results from different field trials, geographical regions, using different agricultural practice etc. Third, in the absence of other more reliable data on genetic stability, differences in gene expression between the "stacked event" and the hybrid may serve as an important indicator. Therefore, parental GM events provide useful information. Given the current practice that often several non-GM and GM hybrids are tested at the same time the additional efforts needed will be limited.

How to properly assess possible interactions of introduced traits

Possible interactions of introduced traits at the nucleic acid, protein and metabolic level are a key issue for „stacked events", as the stacking itself is definitely something that can cause new and unexpected properties. Unfortunately, no methods are readily available to track such interactions and any assessment is largely based on assumptions and indirect evidence. Furthermore, the case studies investigated in this study limited their assessment to the introduced protein and did not consider other levels of interaction.

In the absence of a testing regime that would allow for sound predictions of such interactions, possible adverse health effects can only be identified in compositional analysis or in nutritional feeding studies. Given the limitations of

such studies (see e.g. Spök et al. 2004 for an extensive discussion) it may be reasonable to ask for 90-day whole food toxicity studies.

What would trigger whole-food toxicity studies?

Whole food toxicity studies are not considered a standard requirement by EFSA. Nevertheless an increasing number of recent dossiers include such studies. What would trigger whole food toxicity studies or whether they should be considered a standard requirement in risk assessment is a key issue in a long-standing debate in the scientific literature, among risk assessors and between national Competent Authorities.

In contrast to de-novo assessments of parental GM events, pleiotropic effects of the genetic modification are sometimes considered to be less likely. Provided that the location and expression of the inserts remain intact or at an equivalent level, pleiotropic effects might largely be restricted to possible interactions of the introduced traits at the nucleic acid, protein or metabolic level. However, as mentioned above, due to the inability to test for such interaction and the weaknesses of presently used methods for characterisation, it would be fairly premature to consider whole-food toxicity studies less important than in the case of parental GM events.

With respect to appropriate triggers of whole food studies it is not clear, what guides the decision to request such studies. Currently case-by-case decisions are made claiming they take into account all available evidence. However, it seems that further specific guidance could improve the level of transparency for those decisions. Unfortunately the available documents, including the EFSA Draft Guidance document, do not supply such clarifications that would add predictability for all parties involved.

By way of conclusion, the study presented here shows that some important aspects relevant for "stacked events" are currently not adequately addressed during the risk assessment. Furthermore the proposed guidance by EFSA on the risk assessment of stacked event does not add much further clarification to the current general guidance on assessing GMOs. It remains to be seen how EFSA will use the responses from the public consultation on the draft guidance document when addressing the issues that need to be reviewed.
Further discussions should focus on these open issues and questions and should include a broader range of experts. The objective should be to provide more detailed and scientifically more robust guidance which would be of relevance for applicants, risk assessors and regulatory bodies.

References

ACRE (2004). Scientific safety assessment of GM hybrids. 3 August 2004.

CODEX ALIMENTARIUS COMMISSION (2006). Report of the Fifth Session of the Codex Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology. Ciba, Japan, 19-23 September 2005. Alinomr 06/29/34

EUROPABIO (2005). Safety Assessment of GM crops. Document 5 – Evaluation of crops containing GM events combined by traditional breeding.

DE SCHRIJVER A, DEVOS Y, BULCKE M VAN DEN, LOOSE M DE, REHEUL D, SNEYERS M (2006). Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science & Technology (2006) doi:10.1016/j.tifs.2006.09.002

DOLEZEL, M, ECKERSTORFER, M, GAUGITSCH, H, HEISSENBERGER, A, SPÖK, A (2006). Review of scientific evidence including latest findings concerning Austrian safeguard measures for GM-Maize lines MON810 and T25. Vienna: BMGF. http://www.bmgf.gv.at/cms/site/attachments/9/0/3/CH0255/CMS1161157975708/mon810&t25__bericht_uba&ifz_101006.pdf

EC (2001). Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1-39.

EC (2002). Commission Decision of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L200, 22-33.

EC (2003a). Regulation (EC) No. 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1-23.

EC (2003b). Guidance document for the risk assessment of genetically modified plants and derived food and seed. Prepared for the Scientific Steering Committee by the Joint Working Group on Novel Foods and GMOs. http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf

EFSA (2004a). Letter from EFSA to applicant, dated 27 August 2004, concerning a request for further information for application EFSA-GMO-DE-2004-03 on MON 863 x MON 810 maize submitted under Regulation (EC) No 1829/2003 (Ref. SR/sp (2004) 608).

EFSA (2004b). Guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed. Adopted on 24 September 2004; updated on 7 December 2005; final, edited version of 28 April 2006.

EFSA (2005). Opinion of the GMO Panel on an application (Reference EFSA GMO UK 2004 06) for the placing on the market of insect-protected glyphosate-tolerant genetically modified maize MON863 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Monsanto. Adopted on 6 July 2005 (Question No EFSA-Q-2004-154)

EFSA (2006a). Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing. Draft Guidance. http://www.efsa.europa.eu/etc/medialib/efsa/science/gmo/gmo_consultations/gmo_hybrids_publcons.Par.0001.File.tmp/GMO_hybrids_publconsul.pdf

EFSA (2006b). Opinion of the Scientific Panel GMO on an application (Reference EFSA-GMO-UK-2004-05) for the placing on the market of insect-protected and glufosinate and glyphosate-tolerant genetically modified maize 1507 x NK603, for food and feed uses, and import and processing under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred and Mycogen Seeds. Adopted on 28 March 2006. (Question No EFSA-Q-2004-139), last updated 27 July 2006.

FAGARD M., & VAUCHERET H. (2000). (Trans)gene silencing in plants: how many mechanisms? Annual Review in Plant Physiology and Plant Molecular Biology, 51, 167-194.

LATHAM J.R., WILSON A.K., STEINBRECHER R.A. (2006). The mutational Consequences of Plant Transformation. J. of Biomed. And Biotechnol. Vol. 2006, 1-7.

MONSANTO (2004). Application for authorisation of MON863 x MON810 maize in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed. Technical Dossier.

OECD (2002). OECD guidance for the designation of a unique identifier for transgenic plants. Series on Harmonization of Regulatory Oversight in Biotechnology, No. 23.

SPÖK A, HOFER H, LEHNER P, VALENTA R, STIRN S, GAUGITSCH H (2004). Risk Assessment of GMO Products in the European Union. Toxicity assessment, allergenicity assessment and substantial equivalence in practice and proposals for improvement and standardisation: Vienna: Umweltbundesamt, 2004 Reports Series vol. 253, available at http://www.umweltbundesamt.at/publikationen/publikationssuche/publikationsdetail/?&pub_id=1531; also published as vol. 7-04 in the Series of the Federal Ministry of Health and Women. Vienna: BMGF also available at http://www.bmgf.gv.at/cms/site/attachments/6/8/7/CH0255/CMS1090828056047/risk_assessment_of_gmo_products-bmgf-layout.pdf

SPÖK, A, GAUGITSCH, H, LAFFER, S. PAULI, G, SAITO H, SAMPSON, H, SIBANDA, E, THOMAS W, VAN HAGE-HAMPSTEN, M, VALENTA, R. (2005). Suggestions for the assessment of the allergenic potential of genetically modified organisms. International Archives of Allergy and Immunology 137, pp 167-180.

Annex 1: „stacked events“ in the pipeline

Table 4: Applications for stacked-traits in the EU (status: December 2006)

EFSA-GMO Nr.	Event / Species	Scope of the Application	Current status
UK-2004-01	NK603 x MON810 Maize	Food, feed	EFSA overall opinion published
DE-2004-03	MON863 x MON810 Maize	Food, feed	EFSA overall opinion published
UK-2004-05	1507 x NK603 Maize	Food, feed Import and processing	EFSA overall opinion published
UK-2004-06	MON863 x NK603 Maize	Food, feed Import and processing	EFSA overall opinion published
BE-2004-07	MON863 x MON810 x NK603 Maize	Food, feed Import and processing	EFSA overall opinion published
NL-2005-15	1507 x 59122 Maize	Food, feed Import and processing	Under completeness check** Validation method reports published
UK-2005-17	1507 x NK603 Maize	food and feed, Import and processing, CULTIVATION	Valid application**
UK-2005-20	59122 x NK603 Maize	Food, feed Import and processing	Under completeness check**
UK-2005-21	59122 x 1507 x NK603 Maize	Food, feed Import and processing	Under completeness check**
NL-2005-26	NK603 x MON810 Maize	CULTIVATION	Under completeness check**
NL-2005-28	1507 x 59122 Maize	Food, feed Import and processing CULTIVATION	Under completeness check
UK-2006-29	59122 x NK603 Maize	Food, feed Import and processing CULTIVATION	Under completeness check
UK-2006-30	59122 x 1507 x NK603 Maize	Food, feed Import and processing CULTIVATION	Under completeness check
NL-2006-32	LY038 x MON810 Maize	Food and feed Import and processing	Under completeness check
CZ-2006-33	MON 88017 x MON 810 Maize	Food and feed Import and processing	Under completeness check
NL-2006-35	LLCotton25 x MON 15985 Cotton	Food, feed produced from GM Plants (derived products)	Under completeness check
NL-2005-16	281-24-236 x 3006-210-23 Cotton	Food, feed	Valid application** Validation method reports published
UK-2005-09	MON 531 x MON 1445 Cotton	Food, feed produced from GM Plants (derived products)	Valid application**
UK-2005-10	MON 15985 and MON 15985 x MON 1445 Cotton	Food, feed produced from GM Plants (derived products)	Valid application**

**Application EFSA-GMO-DE-2004-03 is regarded as a new application. Due to the extension of the scope to food and feed aspects a transformation of the application under Regulation (EC) No 258/97 is formally not possible. ** Additional information requested to the Applicant for completeness and/or for risk assessment (by EFSA and/or by JRC-CRL)*

Source: http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html.

Annex 2: Comparison of Guidance Documents

Table 5: Comparative overview of requirements for risk assessment of hybrid GM plants in Guidance Documents and in the scientific literature[b]

Risk assessment studies	**EFSA**	**EUROPABIO**	**Schrijver et al.**
Rationale	Need for further assessment will depend on the nature of the genetic modifications involved	Bridging studies to supplement risk assessment of parental GM events	Additional information
Molecular characterisation	Southern blot and PCR or other appropriate approaches on material representative of commercial cultivars in comparison to parental lines	Fingerprint-type Southern blot analysis in comparison to parental lines; Objective: proof that presence and structure of the inserted material are conserved	Inserts, flanking regions by southern blot; gross structure of inserts; copy number; sequence data irrelevant in case of comparable expression levels
Gene stability	Assess possible changes in trait/phenotype (e.g. herbicide tolerance, expression levels of transgenes compared to parental events	N.r.	Only if part of the F2 progeny[d] will be used for cultivation of the GM stack or the GM stack will be used for GM hybrid production
Gene expression	Assessment of biologically significant changes of expression levels	Limited to raw agricultural commodity (import) or representative issues at plant maturity (cultivation) in comparison to the relevant parental GM events	Compared to parental GM events (to determine need for whole-food toxicity studies)
Toxicity Assessment			
Potential interactions of novel proteins, genes and regulatory sequences	n.sp.[c]	Trigger for additional studies on a case by case basis (proteins)	No tests available
Synergistic toxic effects	Assessed on a case-by-case basis		
Whole-food toxicity study of hybrid[e]	n.sp.[c]	Not required[a]	If expression is increased need would depend on molecular stability

Risk assessment studies	**EFSA**	**EUROPABIO**	**Schrijver et al.**
Whole-food toxicity study of parental lines	n.sp.[c]	Not required	
Novel proteins	Not required if already assessed in parental GM events	Additional studies only if interactions are expected affecting their mode of action	If insert and flanking regions transferred intactly to the hybrid toxicity and in-vivo studies and bioinformatics of parental GM events remain valid
Allergenicity Assessment			
Whole-plant	n.sp.[c]	Not required	"Comparing the allergen repertoire between the non-GM and the GM-stacked event"
Novel proteins	Not required if already assessed in parental GM events	Additional studies only if interactions are expected affecting their mode of action	
Compositional Analysis			
Number of sites	n.sp.	4	
Number of seasons	1 (on case-by case basis: follow-up season)	1	
Geographical Representativeness	Representative to conditions of commercial cultivation	Range of agricultural environments typical of where the crop is grown	
Comparator	"as close as possible" (should be justified)	parental GM events plants or non-GM control of comparable genetic background	parental GM events; non-GM equivalent
Agronomic data	n.sp.	Required similar to above; not further specified	n.sp.
Nutritional Assessment	Trigger for additional studies on a case by case basis	n.sp.	n.sp.
Feeding study	Not explicitly mentioned but might implicitly be covered by case-by-case	On case-by-case basis to confirm wholesomeness	n.sp.

Risk assessment studies	EFSA	EUROPABIO	Schrijver et al.
	approach to the assessment of toxicity, allergenicity and nutritional value		
Environmental aspects	Same as listed in EFSA general guidance plus assessment of environmental exposure levels of effectors	Compositional and agronomic data from typical range of agricultural environments	Assessment of altered potential for interactions with the environment
Detection method	n.sp.	JRC validated detection methods from parental GM events appropriate	n.sp.

Y…yes; n.sp… no requirements specified; n.r…n.required.

a) If no changes to protein mode of action would be expected and if "appropriate animal feeding studies" would be available for all parental GM crops.

b) In general the safety information included in this table are required in case the parental GM events have been assessed already.

c) Trigger for additional studies on a case by case basis.

d) not a common agricultural practice in the EU according to Schrijver et al. 2006.

e) No 90 day whole-food toxicity study was provided / required by EFSA in case of maize NK603xMON810, 1507xNK603 and rape MS8xRF3. In these three cases information already provided was considered sufficient (Schrijver et al. 2006).

Source: EFSA (2006), EuropaBio (2005), Schrijver et al. (2006)

Annex 3: Compositional analysis

Table 6: Composition analysis conducted with hybrid GM plants. (The table includes statistically significant differences across locations)

Events **Analytes**	**Maize 1507 x NK603[b]**		**Maize MON863 x MON810**	
Plant ingredient	**Statistical differences detected**	**Outside literature range**	**Statistical differences detected[d]**	**Outside literature range**
Protein	-		+	-
Fat	+		-	-
Carbohydrates	-		-	-[f]
ADF	-		-	-
NDF	-		+[e]	-
Ash	-		-	-
Moisture[a]	n.d.	n.a.	-	-
Amino acids (18)[a]	+[i]	-[g]	-	-
Fatty acids	[b]		h	
Stearic acid	+[c]	-[g]	+	-
Oleic acid	+	-[g]	-	-
Linoleic acid	+	-[g]	+	-
Palmitic acid	+c	-[g]	+	-
Linolenic acid [a]	n.d.	n.a.	+	-
Arachidic acid [a]	n.d.	n.a.	-	-
Eicosenoic acid [a]	n.d.	n.a.	-	n.d.
Behenic acid [a]	n.d.	n.a.	-	n.d.
Bulk minerals				
Ca	-	-	-	-
K	+[c]	-	+	n.d.
Mg	+	n.a.	-	n.d.
Na	n.d.	n.a.	n.d.	n.a.
P	-	-	-	-
Trace minerals				
Cu	-	-	+	-
Fe	-	-	+	-
Mn	+[c]	-	-	n.a.
Se	n.d.	n.a.	n.d.	n.a.
Zn	+[c]	-	+	-
Vitamins				
Retinolequivalent	n.d.	n.a.	n.d.	n.a.
Vit B1	+	n.a.	-	-
Vit B2	n.d.	n.a..	-	-
Vit B6	n.d.	n.a.	n.d.	n.a.
Vit E	+	n.a.	+	-
Folic acid	+	n.a.	-	n.d.
Niacin	n.d.	n.a.	n.d.	n.a.

Events **Analytes**	**Maize 1507 x NK603[b]**		**Maize MON863 x MON810**	
Plant ingredient	**Statistical differences detected**	**Outside literature range**	**Statistical differences detected[d]**	**Outside literature range**
Others				
Phytic acid	n.d.	n.a.	-	-
Raffinose	n.d.	n.a.	+	-
Furfural	n.d.	n.a.	n.d.	n.a.
Ferulic acid	+	n.a.	-	n.d.
Inositol	n.d.	n.a.	-	n.d.
p-Coumaric acid	+	n.a.	-	n.d.
Trypsin Inhibitor*	n.d.	n.a.	-	n.d.

n.d…data not provided; , n.a. not applicable

a) not included in OECD suggestions.

b) Analysis conducted on grain samples only. Three different herbicide regimes applied: glyphosate, glufonsinate and glyphsate followed by glufonsinate.

c) across locations only; no information about the number and kind of amino acids analysed provided in the Technical Dossier;

d) Proximates and fibres are investigated in both grain and forage. All other analytes are investigated in grain only.

e) in forage only.

f) available for forage only

g) "All mean values within reported literature ranges"

h) Set of 8 fatty acids investigated

i) Methionine, cysteine, aspartic acid.

Annex 4: EFSA draft guidance document „Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing“

Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing

Introduction

Transgenic traits can be combined by conventional plant breeding techniques into new commercial cultivars. This document considers the risk assessment for the commercialisation of plants containing genetic modification events combined by crossing, although the cases likely to be presented for risk assessment will vary according to the type of crop or plant species.

In maize most of commercial crop production is from hybrid seeds, with the exception of some open-pollinated types, for instance the traditional landraces of Mexico and Central America. In order to produce these hybrid seeds, breeders have their own collection of elite parental lines that are used for hybrid production. These lines are produced by crossing a line with itself a number of times (seven or eight at least) in order to be as near as possible to homozygosity or "purity". The hybrid produced will have many of the characters of the parental plants but will also show enhanced properties *e.g.* improved yield that is of interest to farmers. This occurs in the F1 hybrid due to the phenomenon known as heterosis, also referred to as "hybrid vigour".

In the production of genetically modified F1 hybrids, the transformation is carried out in parental lines that are suitable for transformation and regeneration but which may not necessarily be appropriate for commercial seed production. Once the transgenic plant is regenerated it is immediately crossed to one of the elite (higher performance) lines and backcrossing carried out several times to increase the proportion of the genome of the elite maize line as much as possible. The resulting transgenic line may then be used to transfer the event to any other elite line.

If the objective is to combine more than one event in an F1 hybrid, one possibility is to use two elite parental lines, each one with an event, or to cross two versions of the same line with each version having a different transgene. There are many potential variations to the example outlined and which might be used, for example, to combine three or more events.

In species other than maize this process might not be as complex because the use of hybrids is not as extensive. However, in species such as cotton the combination of events may also be produced either in the production of the F1 hybrid or maintained in a single variety. For example, the reproductive propagules planted may be seeds of F1 hybrids, or varieties or may be clonally propagated, vegetative materials such as potato tubers.

Rationale and scope of the document

Where single events have been approved for release into the marketplace, crops produced by crossing the approved GM event with a non-GM parent (a routine approach in plant breeding and using a range of non-GM parental material) are not required to undergo further risk assessment. However, where transgenic events are combined by the interbreeding of existing GM parents, a risk assessment is required as part of the approval process if such combinations are not already covered by the consent for the single events. The need for further assessment will depend, on a case-by-case basis, on the nature of the genetic modifications involved. It is recognized that the genetic background of parents used in the crosses will vary *e.g.* the selection of different elite lines for the

backcrossing process (EFSA's GMO Panel guidance document[1]).

With regard to the assessment of the plants containing genetic modification events combined by crossing the usual starting point is a risk assessment of the single events in parental materials. Clearly where the hybrids contain one or more events not previously evaluated (*e.g.* in parental material) these events require appropriate risk assessment taking into account the requirements of the GMO Panel guidance document.

Key elements of the evaluation process

If the parental GMO events have been assessed by the GMO Panel, the evaluation of the combined events produced from their crossing should concentrate on the following points:

1. Assessment of the intactness of the inserted loci and phenotypic stability

The requirement is to establish that each transgenic locus in the hybrid is the same as in the original independent transformation event. This information will also be important to confirm the identity of samples used in comparative studies, which would include compositional analysis and any trials involving animals. Intactness of loci and comparisons with insert structures in parental lines should be carried out on material which is representative of cultivars produced for commercial production *i.e.* which will enter the environment and the food/feed chain. To assess intactness of loci, applicants should use appropriate molecular approaches *e.g.* Southern blots and PCR analyses and ensure that probes and primers used cover the entire insert and flanking regions.

Stability can be assessed by confirming that the traits targeted by the genetic modification events (the phenotypes) remain unchanged. Changes in the expression of the trait/phenotype might indicate a potential stability issue with respect to the transgenes. For example, in the case of a herbicide-tolerant crop that the plants remain tolerant to the herbicide in question. Any significant change in the expression of the trait/phenotype targeted by the individual transgenes due to the presence of more than one event (*e.g.* events providing tolerance to biotic and abiotic stress) - in comparison to the expression levels of these transgenes in the parental events - should be taken into consideration during the safety assessment, including the environmental risk assessment (ERA). In such cases and where altered expression of the trait/phenotype is viewed as a potential safety issue (see example above) further assessment of the expression levels of the transgenes in plants segregating for the transgene traits is needed.

Applicants should also provide data to indicate the potential for biologically significant changes in the levels of target proteins when traits are combined by crossing. This may have consequences for the ERA.

2. Assessment of potential interactions between combined events

Applicants are requested to carry out a risk analysis on the potential for any interactions between the combined events which could impact on human or animal health and/or the environment.

A step-by-step approach in the risk assessment should be followed:

[1] http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html

1. The risk assessment of the combined events should initially consider the characteristics and properties of each transgenic trait individually. This will also give information, where appropriate, on the segregants from the plants with the combined events.

2. Risk assessment should consider whether or not the occurrence of combined transgenes presents issues that were not addressed when considering the single events *e.g.* in parental lines. For example, the combination of genes may result in altered expression of both endogenous and/or novel traits in a plant.

a) Selection of appropriate comparators

The genetic backgrounds of the controls should be as close as possible to those used in producing the genetically modified plants containing the combined events. The applicant should provide information which validates the choice of controls used for the various parts of the risk assessment.

b) Comparative compositional analysis

Compositional analysis should be carried out alongside appropriate controls grown in the same location and in experiments designed to yield statistically meaningful data. Where the substantial equivalence of parental material containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one years field trialling of events combined by crossing is acceptable where geographical localities are representative of the climatic conditions to which such crops will be exposed. Based on the outcomes of this assessment additional follow-up analysis of compositional characteristics over further growing seasons may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may trigger further assessment.

In terms of the nutrients, anti-nutrients and natural toxins to be analysed the OECD has published a series of consensus documents on the key components that should be considered in the comparative assessment of new crop varieties of particular species. Measurement of these components can be regarded as the minimum requirement for genetically modified events combined by crossing.

c) Assessments of toxicity, allergenicity and nutritional value

This would include, for example, an assessment of any potential for increased toxicity to humans and non-target organism or to modifications in nutritional value due to the combination of the events. This may arise from additive or synergistic effects of the gene products and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This will clearly require a case-by-case approach. The appropriate principles of risk assessment as described in the EFSA guidance document also apply to the assessment of genetic modification events combined by crossing.

d) Environmental risk assessment of plants with combined events

The environmental risk assessment (ERA) will take into account the evaluation of the single events and additional data from molecular characterisation and compositional analysis when determining potential interactions between genes or between gene products. The risk assessment of hybrids will then focus on the possible environmental effects as a result of these interactions. Any new interaction could result in changes to the physiology of the GM plant and related species and

potentially in modified ecological behaviour of these plants. Points to consider for interaction effects within the ERA are the same as those already listed in the EFSA guidance document, *e.g.*:

1. altered toxicity to target organisms and any consequential impact on the development of resistance in target organisms;
2. enhanced toxicity to non-target organisms (including changes in effects on the non- target range);
3. altered fitness of the GM plant or plants acquiring the transgene combination through gene flow;
4. enhanced capacity for gene flow and introgression;
5. altered effects on microbial diversity and activity in relation to biogeochemical cycles.

In addition to the step-by-step approach as described above the following issues need to be considered:

1 Within the framework of field-scale crop production, environmental exposure may be increased to the extent that there could be a significant biological impact which needs to be assessed (*e.g.* for a combination of insect resistance genes producing enhanced levels of toxins).

2 Monitoring plans**:** case specific monitoring should take into account the results of the ERA and any monitoring already established for approved single events. Consideration should be given to any added environmental exposure or effect due to the combination of the events identified in the ERA. General surveillance should be as for any other GM crop and take account of any general surveillance plans already established for the approved single events.

Definitions

Hybrid: The terms hybrid and F_1 hybrid can be defined and used in several ways.

(a) General - The F_1 generation of a cross between two genetically different plants, lines, cultivars, species, or genera.

(b) Specific - A cultivar in which all plants are the F_1 progeny from the crossing of two uniform inbred lines.

F_1's which do not fit in (b) are usually obtained in the process of breeding to obtain new combinations of germplasm, either for maintenance (*e.g.* by vegetative propagation) or for producing an F_2 generation. They may also occur naturally. Hybrids that fit into category (b) and which are produced for use as a commercial crop as the F_1 generation must be newly produced for each new seed crop.

Inbred Line: A line produced by continued inbreeding. In plant breeding an inbred line is a nearly homozygous line usually developed by continued self-fertilization, accompanied by selection.

Pure line: A strain homozygous at all loci, ordinarily obtained by successive self-fertilizations in plant breeding.

Isogenic lines: A group of individuals that possesses the same genotype, irrespective of their being homozygous or heterozygous.

Near-isogenic lines:	A group of lines that are genetically identical except at one or a few loci.
Recurrent parent:	The parent to which successive backcrosses are made in backcross breeding.
Backcross:	A cross of a hybrid to either of its parents. In genetic terms a cross between a heterozygote and a homozygous recessive. In wild inter-specific or intraspecific hybrid plants it is a cross with either of the parental types as a first step towards introgression of genes into populations of the parental type.
F_1:	The first generation of a cross.
F_2:	The second filial generation obtained by self-fertilization or crossing *inter se* of F_1 individuals
F_3:	Progeny obtained by self-fertilizing F_2 individuals.
S_1, S_2, S_3, . . .:	Symbols for designating first, second, third, *etc.*, self-fertilised generations from the ancestral plant designated as S_0.
Heterosis	Heterosis, also known as "hybrid vigour", describes a situation where crossing two inbred lines yields progeny (F_1 hybrids) that are, for example, more healthy or vigorous than their parents or which fall outside the range of the parents with respect to other characteristics.
Locus:	The position that a given gene occupies in a chromosome.
Transgene locus:	The position occupied by the inserted (transgenic) construct in a chromosome.

Annex 5: IFZ-Comments on EFSA Draft Guidance for risk assessment of "stacked events"

Preliminary remarks

In July 2006 EFSA published its Draft Guidance "Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing", subsequently designated as the "Draft Guidance", on its website and asked for comments. This commentary includes suggestions for further refinement of this Draft Guidance. It focuses on aspects of toxicity and allergenicity assessment and on composition analysis only. Other areas are not covered. This commentary builds on an earlier much more extensive commentary on the EFSA Guidance on GMO risk assessment (SPÖK et al. 2004a, EFSA 2006).
The comments below have been submitted on 7 Sept. 2006 to EFSA using the web tool available at http://www3.efsa.europa.eu/cf/consultation.cfm?doc=11.

Comparative compositional analysis

> 115 "Compositional analysis should be carried out alongside appropriate controls"

Comment: Crop-specific examples for should be given to illustrate what would be considered as appropriate non-modified controls. Controls should also include the GM-modified parental plants.

> 116-123: "Where the substantial equivalence of parental material containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one years field trialling of events combined by crossing is acceptable where geographical localities are representative of the climatic conditions to which such crops will be exposed. Based on the outcomes of this assessment additional follow-up analysis of compositional characteristics over further growing seasons *may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may trigger further assessment* (Draft Guidance)."

Comment:
Dealing with (statistically significant) differences: According to the understanding of the author of this commentary, this seems to imply that statistically significant differences might only be further investigated if they would fall outside natural variation. Thereby the importance of such differences for guiding further investigation is even weakened compared to the EFSA Guidance document on GMO risk assessment of 2004 (updated version: EFSA 2006)[4]. This appears to be a further shift in EFSA's interpretation of the concept of substantial equivalence as there is even no need to examine significant differences that fall outside normal ranges. The compositional analysis of maize MON863xMON810 conducted for application under Regulation 1829/2003 included 71/56 significant difference to MON846/to MON863, 122 significant differences to MON810 and 142 significant differences to commercial lines of a total of 290 comparisons. Furthermore, large differences have been observed in expression levels of CRY3Bb1 in MON863 between different field trials. Such differences would clearly

[4] **Statistically significant differences between parental and GM lines, which are not due to the intended modification, may indicate the occurrence of unintended effects, and should be assessed specifically with respect to their safety, nutritional impact and environmental implications.(p 13-14) Statistically significant differences in composition between the modified crop and its non-genetically modified comparator grown and harvested under the same conditions should trigger further investigations as to the relationship between the identified difference and the genetic modification process. Modifications that fall outside normal ranges of variation will require further assessment to determine any biological significance. (p 24).**

ask for further investigation. According to the Draft Guidance, these investigation would not be required if ranges are still within the normal variation.
Pursuing this approach would also put more importance on the database of natural variation. According to the experience of the author of this commentary, literature data frequently cited in the dossiers are sometimes drawn from rather old and possibly outdated papers and the very different methods used to generate those data and the different quality of the data are usually not considered (SPÖK et al. 2004b).
In the absence of reliable and internationally acknowledged database of crops-specific normal variation and in accordance with an understanding of compositional analysis as providing *indications* for unintended effects, I propose that in case of significant differences analyses should be repeated and the set of parameters should be extended.
Representativeness of field trials: It should also be stated clearly, that in any case field trials should be conducted on a set of geographic locations that would be representative for the commercial cultivation intended. Applicants should include evidence that links the selected set of field locations to the main areas of future commercial cultivation. According to variability and influence of differences in climatic conditions, field trials should also not be limited to just one year.

Assessments of toxicity, allergenicity and nutritional value

> 132-138: "This would include, for example, an assessment of any potential for increased toxicity to humans and non-target organism or to modifications in nutritional value due to the combination of the events. This may arise from additive or synergistic effects of the gene products and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This will clearly require a case-by-case approach. The appropriate principles of risk assessment as described in the EFSA guidance document also apply to the assessment of genetic modification events combined by crossing." (Draft Guidance)

Comment:
On toxicity assessment: The only acknowledged synergistic effects are between the newly introduced gene products. While I would agree that this kind of effects be particularly considered, this does not mean that more general synergistic effects, for instance originating from interactions of the introduced genes/proteins an its genetic/metabolic context would not be important.
If this possibility of synergistic effects would be truly recognised, the Guidance should clearly ask for 90-day sub-chronic feeding studies of the whole GM crop - in accordance with the EFSA Guidance on GMO risk assessment (EFSA 2006).
The way EFSA has dealt with „stacked events" included checking for possible interactions of the inserted genes or proteins. In fact results from metabolomics indicate that connectivity between pathway might be much higher than thought previously. E.g. in Saccharomyces cerevisiae about 50% of the metabolites are involved in more than two reactions (NIELSON & OLIVER 2005). However, so far it has been sufficient to make plausible that both genes/proteins and the pathways where they do play a role are not related and interactions unknown. In essence this could mean to simply dismiss this interactions on the basis of absence of evidence. Thus, de-facto ignorance might be considered a safety argument.
The only methods presently available to risk assessment are whole-plant toxicity studies. Although, both the conduct of such studies and the interpretation of results might not be that clear-cut, there is an increasing experience with this

kind of studies including proposals of how to conduct this kind of studies (PUSZTAI et al. 2003). In fact, EFSA asked for 90-day whole-plant feeding studies despite the existence of such studies on the parental GM plants (e.g. maize MON863xMON810, MON863xNK603, MON863xMON810xNK603 but not in case of maize 1507xNK603).
In the absence of acknowledged profiling methods it is thus proposed that the Guidance should ask for 90-day whole food studies conducted under GLP and according to internationally recognised guidance as a *standard* requirement. This type of studies should also be required if such studies had already been conducted on the parental GM crops.
On allergenicity assessment: Allergenicity assessment of the introduced protein should be complemented by an assessment of the whole-plant as described in SPÖK et al. 2005. Many plant allergens belong to a family of pathogenesis related proteins, the expression of which could be upregulated by infections, hormones and other stressors (HANNINEN et al., 1999; BREITENEDER et al., 2000; HOFFMANN-SOMMERGRUBER, 2002; MIDORO-HORIUTI et al., 2001). Whole-plant studies such as those proposed in SPÖK et al. (2005) would also consider other important exposure routes that have been previously dismissed by the GMO panel (inhalation of pollen and dust e.g. during handling and processing of the plants). However, GM crops may exhibit allergenic activity also via other routes, particularly when they are grown on a large scale and processed by different means. For example, pollen represents a more potent and frequent allergen source than plant-derived food and it should, therefore, be considered that GMPs may also release allergens via pollen production and hence cause respiratory sensitization. Furthermore, processing of maize may lead to respiratory sensitization in bakers who are exposed to flour. In this context it has been reported that soybean dust caused severe outbreaks of asthma in Barcelona, Spain when the soybeans were unloaded in the city harbour (CODINA et a. 1999, ANTO et al. 1993). Another example for respiratory sensitization has been described in employees who are exposed to papain (NOVEY et al. 1980). The cross-reactivity of IgE antibodies of papain with latex allergens has been reported using sera of latex-exposed and papain-exposed people (BAUR et al. 1995). Hypersensitivity to papain was found in approximately 1% of an allergic population using skin prick tests and IgE measurements and was confirmed by oral challenge (MANSFIELD et al. 1985). We, therefore, suggest that other exposure and sensitization scenarios be considered when assessing GMPs or other products rather than focusing on the gastrointestinal route only.
Protein expression: Requirements for data for protein expression are not mentioned in the Draft Guidance. Protein expression is an important prerequisite for exposure assessment. Differences in expression of target proteins between the hybrid and the parental plants could also be a valuable indicator for unintended effects. As such differences are actually documented in recent applications for „stacked events" (e.g. from the Directive 2001/18/EC Dossier of maize NK603xMON810) it might therefore be beneficial to explicitly include this as a requirement in the Draft Guidance.

References

ANTO JM, SUNYER J, REED CE, SABRIA J, MARTINEZ F, MORELL F, CODINA R, RODRIGUEZ-ROISIN R, RODRIGO MJ, ROCA J, SAEZ M: Preventing asthma epidemics due to soybeans by dust control measures. N Engl J Med 1993; 329: 1760–1763.

BAUR X, CHEN Z, ROZYNEK P, DUSER M, RAULF- HEIMSOTH M: Cross-reacting IgE antibodies recognizing latex allergens, including Hev b1, as well as papain. Allergy 1995; 50: 604–609.

BREITENEDER, H. & EBNER, C. (2000): Molecular and biochemical classification of plant-derived food allergens. Journal of Allergy and Clinical Immunology 106: 27-36.

CODINA R, LOCKEY RF, FERNÁNDEZ-CALDAS E, RAMA R: Identification of the soybean hull allergens responsible for the Barcelona asthma outbreaks. Int Arch Allergy Immunol 1999; 119: 69–71.

EFSA (2006) Guidance document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed. Adopted on 24 September 2004; updated on 7 December 2005; final, edited version of 28 April 2006.

HANNINEN, A.R., MIKKOLA, J.H., KALKKINEN, N., TURJANMAA, K., YLITALO, L., REUNALA, T. & PALOSUO, T. (1999): Increased allergen production in turnip (Brassica rapa) by treatments activating defense mechanisms. Journal of Allergy and Clinical Immunology 104: 194-201.

HOFFMANN-SOMMERGRUBER, K. (2002): Pathogenesis-related (PR)-proteins identified as allergens. Biochemical Society Transactions 30: 930-935.

MANSFIELD LE, TING S, HAVERLY RW, YOO TJ: The incidence and clinical implications of hypersensitivity to papain in an allergic population, confirmed by blinded oral challenge. Ann Allergy 1985; 55: 541–543.

MIDORO-HORIUTI, T., BROOKS, E.G. & Goldblum, R.M. (2001): Pathogenesis-related proteins of plants as allergens. Annals Allergy Asthma Immunology 87: 261-271.
NIELSEN, J. and OLIVER, S. (2005) The next wave in metabolome analysis. Trends Biotechnol. 23, 544–546

NOVEY HS, KEENAN WJ, FAIRSHTER RD, WELLS ID, WILSON AF, CULVER BD: Pulmonary disease in workers exposed to papain: Clinico-physiological and immunological studies. Clin Allergy 1980; 10: 721–731.

PUSZTAI, A, BARDOCZ S, EWEN, SWB (2003) Genetically Modified Foods: Potential Human Health Effects. In: Food Safety: Contaminants and Toxins (ed. JPF D'Mello) pp.347-372. CAB International, Wallingford Oxon.

SPÖK, A, GAUGITSCH, H, LAFFER, S. PAULI, G, SAITO H, SAMPSON, H, SIBANDA, E, THOMAS W, VAN HAGE-HAMPSTEN, M, VALENTA, R. (2005): Suggestions for the assessment of the allergenic potential of genetically modified organisms. International Archives of Allergy and Immunology 137: 167-180.

SPÖK, A., SCHMATZBERGER, A. & GAUGITSCH, H. (2004a): Commentary on the „Draft Guidance Document for the risk assessment of genetically modified plants and derived food and feed. Vienna, Graz: Umweltbundesamt, IFZ, May 2004.

SPÖK, A., HOFER, H., LEHNER, P., STIRN, S., VALENTA, R., AND GAUGITSCH, H. 2004b. Risk assessment of GMO products in the European Union .Toxicity

assessment, allergenicity assessment and substantial equivalence in practice and proposals for improvement and standardisation. Series Berichte 253. Vienna: Umweltbundesamt.
http://www.umweltbundesamt.at/fileadmin/site/publikationen/BE253.pdf. Also published as Volume 7-04 in the Series of the Bundesministerium für Gesundheit und Frauen, Vienna, available at
http://www.bmgf.gv.at/cms/site/attachments/6/8/7/CH0255/CMS1090828056047/risk_assessment_of_gmo_products-bmgf-layout.pdf.

Annex 6: Umweltbundesamt-Comments on EFSA Draft Guidance for risk assessment of stacked events

Comments on EFSA Document „Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing“

EFSA Public consultation, deadline 10.09.2006

General

The Umweltbundesamt Wien (Federal Environment Agency Vienna, Austria) welcomes that EFSA gives the opportunity to interested parties and stakeholders to provide comments to the above mentioned draft document.

A guidance document in order to address the crucial points in risk assessment of GM plants containing genetic modifications combined by crossing certainly is needed. However, the draft document at hand is very vague, and adds little to the risk assessment criteria already laid down in the "Guidance Document on risk assessment of GM Plants and derived food and feed" published by EFSA in 2004. In addition to that the purpose of the document remains quite unclear as it is lacking crucial information in order to be a guidance document for applicants. There is no specific information provided on the data required, the methods how to generate these data and the assessment parameters and endpoints, nor is there any information on how to assess these.

Introduction

The main part of the introduction focuses on basics of maize breeding. Though it is recognized that at the moment maize is the most important crop containing genetic modification events combined by crossing, this is in our opinion much too specific for a guidance document which is not specifically focused on maize.

Line15: The term "purity" is in plant breeding usually used in the context of seeds and not referring to homozygosity. As the term is not needed for a better understanding it should be omitted.

Line 18: Heterosis is just one effect which might occur. There is no reference to pleiotropic effects, which might also occur, and in the context of risk assessment of GMOs are of much higher importance.

Lines 32 to 36: This paragraph is completely unclear, and badly written. E.g. it refers to cotton and mentions potato tubers as an example in the next sentence. There seems also to be a mix of terminology: e.g. "varieties" are mentioned in

the same context as seeds, i.e. as planting material, which according to plant breeding terminology is wrong.

Line 35: Propagules are vegetative portions of a plant, such as a bud or other offshoot, that aid in dispersal of the species and from which a new individual may develop. Therefore seeds cannot be propagules.

Rationale and scope of the document

Line 46: We think that there is always a need for further assessment of GM plants which contain genetic modification events combined by crossing. By simply evaluating the parental lines, effects like heterosis (which is mentioned as the main effect in the introduction of this document) and pleiotropic effects cannot be assessed.

Line 52: The risk assessment of the parental GM events is stated to be the starting point of GM plants which contain genetic modification events combined by crossing. There is no information about the next steps, which are - in our understanding - the main scope of this document.

Lines 53 to 55: Here it is stated that only hybrids containing one or more events previously not evaluated should undergo appropriate risk assessment. We, on the contrary, believe that an appropriate risk assessment should always be done. The definition of an "appropriate" risk assessment is currently under discussion and needs to be further developed. In this context no specific information is given by the document.

Key elements of the evaluation process

1. Assessment of the intactness of the inserted loci and phenotypic stability

Line 63 (header): A locus (see also "definitions") is the position of a gene. Therefore the assessment of the "intactness of the inserted loci (=positions)" is wrong terminology. It can be assessed if the inserted gene is intact, and if it is still in the same position in the genome as in the parental line.

Line 70: see comment on line 63.

Line 71: In the list of adequate molecular approaches to characterisation only the techniques Southern blotting and PCR-Analysis are listed. Sequencing the most specific method to assess the intactness of the construct and the border sequences is not mentioned in the document.

Lines 74 to 84: this paragraph exclusively refers to quantitative effects, i.e. the level of expression of the proteins. There is no mentioning of the assessment of possible qualitative effects.

Lines 86 to 88: This statement is very general. Indication on how this potential should be assessed and what might be the possible significant changes should be given.

2. Assessment of potential interactions between combined events

Lines 98/99: It is unclear what "appropriate" means in this context. What is the information which can be derived from the evaluation of the parental GM events, relevant for plants with the combined events?

Lines 101 to 104: In principle the content of this paragraph is correct. However, it is only a very vague outline on what should be done by the applicant. Neither in the paragraph itself nor in the following text, which also remains very general, details are given on parameters, methods and endpoints.

Lines 103 and 104: If this paragraph is meant to be a general description of the second step of risk assessment, it is unclear why there is an example given, which is only one of many issues to be addressed. In our opinion this may indicate a preference in the assessment, leading to neglecting other important issues.

a) Selection of appropriate comparators

Lines 109 to 111: An indication should be given on what controls should be used in the assessment: The GM parental lines or genetically very close non-GM lines? In the last sentence of this paragraph it is suggested that the applicant may use different comparators for different parts of the risk assessment. This should be clarified, because the comparability of data strongly depends on the use of controls, and therefore the risk assessment may be jeopardized by choosing different controls, even if information on the reasons for the choice by the applicant is given.

b) Comparative compositional analysis

Lines 116 to 120: In this sentence it is stated that an assessment of the parental lines will lead to a limitation of field trials of plants with the combined events to 1 year. Regarding that there is no scientific justification given, we believe that there should be no *a priori* limitation to the extent of required field trials. The duration and number (which is not addressed in the document at all) has to be decided on a case by case basis.

Lines 121: In addition to extend the duration of the field trial it might also be necessary to extend the number of parameters, in order to investigate the cause of the unexpected changes in the compositional characteristics. This is not addressed in the document.

c) Assessments of toxicity, allergenicity and nutritional value

Line 132: The assessment of "…increased toxicity to humans, and non-target organisms …" should not be mentioned as an example! This is one of the main elements of risk assessment.

Lines 136 to 138: It is clearly stated here that the risk assessment requires a case-by-case approach and that the general principles of risk assessment should be followed also for plants containing genetic modification events combined by crossing. We believe that this is a very valid and important statement and should apply not only for the assessment of toxicity, allergenicity and nutritional value, but for the whole risk assessment. We would therefore recommend shifting it into the scope of the document.

d) Environmental risk assessment of plants with combined events

The whole clause is very general and basically repeats statements given earlier in the document and contents from the Guidance Document on Risk Assessment (EFSA 2004b). Again more details are required, if this document should give guidance to the applicants.
In addition there is some contradiction to other parts of the document as some parameters mentioned in this clause (introgression, altered fitness of plants acquiring the transgene combination through gene flow, …) can not be assessed in a one year field trial (as requested in line118).

Definitions

Some definitions are not needed, because the respective terms do not appear in the text. Namely: Line 193 "pure line", line 195 "isogenic lines", line 198 "Near-isogenic lines", line 199 "recurrent parent", line 209 "F3", line 210 "S1, S2, S3, …"

Lines 176 to 189: This is not really a definition, but a collection of examples. For the purpose of the document the definition given under "(a)" is sufficient.

Lines 190 to192: An inbred line is not "usually" developed by self-fertilization. An inbred line is also achieved by repeated crossing close relative plants or (laboratory) animals, which are not self-fertilizing.

In den letzten Jahren wurde in der EU eine steigende Zahl von genetisch veränderten Pflanzen (GVP) mit kombinierten Eigenschaften (“stacked events”) zur Zulassung nach der Richtlinie 2001/18/EG bzw. der Verordnung (EG) No. 1829/2003 angemeldet. Kontroversen über die Zulassung von “stacked events” und insbesondere über die Erfordernisse für die Risikoabschätzung bei “stacked events”, haben einen hohen Klärungsbedarf angezeigt. Die vorliegende Studie wurde durchgeführt, um Schlüsselelemente für die Risikoabschätzung von “stacked events” zu identifizieren und zu untersuchen, wie diese in der regulatorischen Praxis bei der Risikoabschätzung von bestimmten GVP berücksichtigt werden.