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CHAPTER_TEXT	COMMENT_TEXT
OTHER SCIENTIFIC COMMENTS	Annex III is dedicated to give the format of technical dossiers. It should be modified according to the modifications proposed in the previous sections.
7.6. Conclusion of the toxicological/nutritional and allergenicity assessment	Line 1835: we suggest a new bullet point: “whether the improvement in nutrient content and its availability in target animals are effective”. Line 1843: after additional, insert “compositional”. Overall comment on toxicological assessment : In the EFSA approach, the toxicological testing of the whole GM plant is linked to the results of the comparative assessment. If the comparative assessment does not identify differences, there is no need to conduct further toxicological testing of the whole GM food, however the newly expressed proteins should be submitted to toxicological testing. If the comparative assessment identifies new constituents, they should be tested like the newly expressed protein, but not the whole GM food, at the exception of certain cases. Therefore, the EFSA strategy regarding the safety assessment of GM plants is essentially driven by the application of the concept of equivalence. XXX does not share this approach, as developed in a previous AFSSA opinion (AFSSA2008). Briefly, the French Agency recommends carrying out a 90-day toxicity study for any new genetic event. A toxicity study, however, is not required with genetically modified plants containing stacked transformation events, when a 90 day toxicity test has already been performed as part of the parental GMO assessment and deleterious effects have not been observed. It is thus proposed that EFSA bases the safety assessment of GM plants on the study recently published by Knudsen and Poulsen, i.e. the SAFOTEST approach (Knudsen and Poulsen, 2007). “The safety assessment in SAFOTEST is drawing both on the knowledge about the identity of the genetic change, the compositional data of the GM food and the 90-day toxicity study on the GM food with and without the spiked material, before the hazard characterization is concluded”. References : AFSSA 2008, avis de l’AFSSA relatif aux études de toxicité réalisées dans le cadre des demandes de mises sur le marché d’OGM”, 29 fevrier 2008. Knudsen I and Poulsen M, 2007, Comparative safety testing of genetically modified foods in a 90-day rat feeding study design allowing the disctinction between primary and secondary effects of the new genetic event”, Regul. Tox. And Pharma., 49, 53-62.
7.5. Anticipated intake/extent of use	Line 1814: please after other, add “specific or”

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7.4.2. Nutritional assessment of GM feed	Line 1765: at the end of the line, we suggest indicating: ”this analysis should also concern mycotoxin and pesticide contents (ILSI 2003). “ Line 1768: please insert “or laying cycle for laying hens or quails” after “dairy cow” Line 1772: please add “content and “ after improved Line 1775: after “varieties”, we suggest adding the following sentences: “in case of herbicide tolerant GM plants, plant use for the diet formulation should be exposed to the intended herbicide. Line 1777: please add “or a vitamin” after “amino acid”. Line 1798: we suggest adding peer-reviewed articles references such as: Emmert and Baku, 1997, J. Applied Poultry Research, 6, 462-470. Baker and Kan, 1994, Poultry Science, 73, 1441-1447.
7.4.1. Nutritional assessment of GM food	Line 1721: please add “ toxicants” after antinutrients. Line 1753: after “basis”, the publications mentioned lines 1798-1799 section 7.4.2 on bioavailability of nutrients might be referenced.
7.3.1. Assessment of allergenicity of the newly expressed protein	Concerning the proposed use of serum bank, we wonder about the availability of serum bank to allow serum-binding assay and targeted serum screening and consequently the feasibility of these tests.
7.2.5. Toxicological testing of the whole GM food/feed	Line 1448: Title of this section should be: 7.2.5. Toxicological testing of the whole GM food / feed and / or food / feed derived from the GM plant. Lines 1449 to 1457: 1450: We suggest like in the introduction 7.2 (line 1335) adding “a comprehensive” to compositional analysis. The correct sentence would be: “The risk assessment of the GM plant and derived food/feed is primarily based on molecular characterization, comparative agronomic, phenotypic and a comprehensive compositional analysis, and the toxicological evaluation of the identified intended and unintended effects.” 1457: We suggest adding a third bullet point as follows: “(iii) toxicological testing of whole GM food/feed is mandatory to assess the potential occurrence of unintended and / or unpredicted effects as it could be the case for a new genetic event (see AFSSA, 2008)”. AFSSA 2008, avis de l’AFSSA relatif aux études de toxicité réalisées dans le cadre des demandes de mises sur le marché d’OGM”, 29 fevrier 2008. Lines 1458 to 1461: Please delete from “based on the preceding….” to the end of the sentence and add “and / or unpredicted” before “effects”. The corrected sentence would be: “Furthermore, toxicological testing of whole GM food/feed should be considered if there are any indications or remaining uncertainties on the potential occurrence of unintended and / or unpredicted effects.” Line 1466: “including analysis of immunotoxicity.” should be added after “(OECD, 1998)” Lines 1519 to 1572: Interpretation and relevance of toxicity tests This section should be deleted and included as an appendix.
7.2.2. Toxicological testing of	Lines 1375-1377: the concept of “history of safe consumption by humans and animals” should be clearly defined and supported by both qualitative and quantitative data, see Constable et al., 2007.

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newly expressed proteins	Constable A, Jonas D, Cockburn A, Davi A, Edwards G, Hepburn P, Herouet-Guicheney C, Knowles M, Moseley B, Oberdörfer R, Samuels F. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chem Toxicol. 2007 45, 2513-2525. Lines 1388 to 1409: The sentence should be completed as follows: "To demonstrate the safety of newly expressed proteins without any significant history of safe human consumption:" and a first bullet point related to "acute toxicity testing" should be added. Lines 1410 to 1418 should be deleted We do not understand the rationale of such studies for the following reasons: 1- the aforementioned set of data is sufficient to correctly assess newly expressed proteins, 2- the newly expressed protein is not consumed as such. It is part of GM plant where it may potentially interact with other constituents. Such interactions may be of safety concern. Therefore, the whole food should be tested, not the sole protein.
7.2.1. Standardized Guidelines for Toxicity Tests	Lines 1364-1366: this paragraph should not appear in this section and would be more appropriate in the next section that is dedicated to the toxicological testing of newly expressed proteins. The results of an acute test, i.e. the single dose exposure should not be used to assess the repeated exposure. However, in the case of newly expressed proteins for which there is no available safety data, such a test is of value since it is the basic tool used in hazard identification.
7.2. Toxicology	Lines 1335-1336: we suggest adding "a comprehensive" after "outcome of" to focus on the occurrence of unintended effect as stated in section 2.2, lines 598-615. The sentences would be as follows : "The requirements of toxicological testing must be considered on a case-by-case basis and will be determined by the outcome of a comprehensive comparative analysis, i.e. the" Lines 1345-1347: we suggest replacing the last sentence by: "In such cases, the tests proposed by the applicant must be validated. Furthermore, the applicant must state the reasons for not submitting the required studies or for carrying out other than those mentioned below".
7.1.7. Conclusion of the comparative analysis	Line 1299: at the end of the line in third bullet point, insert: "Whether average values issued from compositional analysis are in the range of values published in international feed tables, OECD, ILSI, NRC and /or European tables". Line 1310: We suggest adding the following comment: "Data of the comparative analysis of composition should be compared with those issued from nutritional equivalence analysis for target animals".
7.1.6. Effect of processing	Line 1262: please replace "soya" by "oil seed meals"; Line 1266: insert "The different chemical and physical processes should be described such as duration and nature of the treatments" Line 1291: add "The effect of processing on the expressed protein(s) and possibly on the level of some antinutrients and toxicants (e.g. gossypol, antitryptic factors, glucosinolates…) should be indicated
7.1.4. Comparative analysis of composition	Line 1211: the reference (OECD a) is not sufficiently informative, we suggest adding "consensus document on compositional document prepared by the Internal Coordination Group for Biotechnology includes specific recommendations for low erucic acid rapeseed (canola), soybean, sugar beet, potatoes, maize, bread wheat, rice, cotton, barley, alfalfa, sunflower, then papaya, cassava and sweet potatoes (see www.oecd.org/dataoecd/33/1/40628456.pdf)"

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	Line 1224: we suggest inserting “antinutrients”, after key and “or deleterious” after toxic. Line 1227: at the end of the line, we suggest inserting “Grains and derivatives should be tested for specific mycotoxins that can affect animal health (see ILSI 2003). The pesticides residues to be evaluated should be determined by those sprayed on the crop”; according to the Directive 91/414 annex I inclusion decision Line 1241 : we suggest inserting the following sentence “Appropriate analytical methods assessed internationally are highly recommended such as Official Methods of Analysis of AOAC International (2000). Official Methods of Analysis of AOAC INTERNATIONAL (2000) 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 999.11. “
7.1.3. Selection of material and compounds for analysis	Lines 1194- 1203: we suggest inserting in this part, a mention on sampling procedures: “the applicant should follow recommendations on sampling procedures, e.g. regarding representative samples (grain, hay, derivatives, etc), these recommendations are described in the following references: ILSI, 2003, Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits. 2004/787/EC, Commission Recommendation on technical guidance for sampling and detection of GMOs and material produced from GMOs as or in products in the context of Regulation EC 1830: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2004/l_348/l_34820041124en00180026.pdf CEN/TS 15568/2007 Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products – Sampling strategies.”
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Line 1037: we suggest writing “at least 2 years” instead of “many years”. Line 1062: we suggest adding this general comment: experimental design must be appropriate for an interpretation with the proposed model described under (c) statistical analysis (see line 1082). Line 1070: the number of the herbicide‘s applications should be given. Line 1111: at the end of the line, we suggest inserting the following sentences: “Comments should be particularly focussed on average values for combined sites, number of replicates per site and years should also be indicated. In case of missing data, appropriate comments should be given”.
7.1.1. Choice of the comparator	Line 971 after “where a” “specific” should be added. Line 975 : replace “composition” by “chemical composition”. Lines 983-988: this part should be deleted up to “these events” and replaced by the following sentence: “In the case of events stacked by conventional crossing, a single assessment for the highest number of stacked events could cover all combinations with fewer of these events”.
4. Genetic stability of the insert and phenotypic stability of the GM plant	Line 920: Stability could also be investigated over various genotypes or generations of backcross. Line 922: The case of multiple insertions from a single event should also be considered and especially if these insertions are not genetically linked and if only some of them (that do not necessarily hold the transgene responsible for the trait) are not stable.
4. Genetic stability of the insert and phenotypic stability of the GM plant	Line 920: Stability could also be investigated over various genotypes or generations of backcross. Line 922: The case of multiple insertions from a single event should also be considered and especially if these insertions are not genetically linked and if only some of them (that do not necessarily hold the transgene responsible for the trait) are not stable.
3. Information on the	Line 896: When the transgene product is RNAi and acts as a gene regulation element, a whole genome in silico analysis should be carried out to verify that no

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expression of the insert	other gene than the target gene could be affected by the genetic modification. Line 898: Quantitative detection methods like Q-RTPCR and mass spectrometry should be used preferentially. Line 899: A mass spectrometry analysis of the recombinant protein in planta should be provided.
2. Information on the sequences actually inserted or deleted	Line 840 The complete sequence of the inserts is clearly required in order not to only check the transgene integrity but also to control in silico that the expected protein will be synthesized, thus avoiding protein purification from the plant. Precisions about the antibiotic corresponding to the antibiotic resistance marker gene (ARGM) should be given and described according to EFSA, 2004, Opinion of the scientific panel on GMO on the use of ARMG as marker gene in genetically modified plants, EFSA Journal, 48,1-18. Line 850: Chromosomal localisation should be provided whenever possible. This is necessary in order to better characterise of the environment surrounding the insertion site and to identify distant regulation regions. Line 856: This sequence should cover at least 1 kb in both 5' and 3' of the insertion site. Line 859: A bioinformatic analysis of the re-associated 5' and 3' flanking regions should be provided in order to better identify the insertion site. Line 864. A systematic RT-PCR detection of the RNA encoding putative new ORF should be carried out. Line 866. Micro RNA are now known to play an important role in gene regulation and more and more interaction sites for micro RNA are known. A bioinformatic analysis should be provided in order to identify putative new micro RNA or interaction sites.
1. Description of the trait(s) and characteristics which have been introduced or modified	Line 835: When genetic modification leads to an herbicide tolerance, applicants should provide information on herbicide's mode of action and a description of the active substance metabolism in the plant. The authorization status of the pesticide should be clarified at the European level and worldwide. When genetic modification leads to an insect resistance, applicants should provide information on the structure and mode of action of the insecticide protein.
OTHER SCIENTIFIC COMMENTS	2306-2312 It is very unclear how monitoring of health will be undertaken. It is widely accepted in the medical community that current surveillance mechanisms are inadequate to monitor common illnesses such as diabetes and asthma. Monitoring of adverse ill-effects from genetically engineered foods must include human biochemical monitoring as the development of symptoms is a late development in disease pathology
7.2.2. Toxicological testing of newly expressed proteins	1375-1378 It is unclear how a "history of safe consumption" is validated as no surveillance systems are currently in place by which this statement can be supported. Anecdotal reports of increased incidences of allergies to soya have not been investigated on a scientific basis.
7.2. Toxicology	Untersuchung auf gesundheitliche Risiken Wie der Fall des gentechnisch veränderten Mais MON863 zeigt, sind die derzeitigen Standards der Risikobewertung nicht nur im Hinblick auf ihre statistische Auswertung strittig (Séralini, G-E, Cellier, D. & Spiroux de Vendomois, J. 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Archives of Environmental Contamination and Toxicology.). Der Fall einer Erbse, die mit einem Eiweiß (Amylase) aus der Bohne verändert wurde (Prescott VE, et al, 2005, „Transgenic expression of bean a-amylase inhibitor in peas results in altered structure and immunogenicity", J

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	Agricultural and Food Chemistry, 53, 9023-30.), zeigt, das aus gentechnisch veränderten Pflanzen erhebliche gesundheitliche Risiken resultieren. Eine vertiefte Analyse des Falles zeigt (Valenta, R. & Spök, A., 2008, „Immunogenicity of GM peas“, BfN Skripten 239, Bundesamt für Naturschutz, Bonn.), dass die derzeitigen Standarduntersuchungen, wie sie auch von der EFSA vorgesehen sind, in diesem Fall kaum geeignet gewesen wären, dieses Risiko zu erkennen. EFSA muss aus den Fällen wie MON863 und der Amylase-Erbse umfassende Konsequenzen für die eigenen Vorgaben ziehen. Das ist in der jetzt präsentierten Vorlage in keinster Weise erfolgt.
7.3. Allergenicity	Untersuchung auf gesundheitliche Risiken Wie der Fall des gentechnisch veränderten Mais MON863 zeigt, sind die derzeitigen Standards der Risikobewertung nicht nur im Hinblick auf ihre statistische Auswertung strittig (Séralini, G-E, Cellier, D. & Spiroux de Vendomois, J. 2007. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Archives of Environmental Contamination and Toxicology.). Der Fall einer Erbse, die mit einem Eiweiß (Amylase) aus der Bohne verändert wurde (Prescott VE, et al, 2005, „Transgenic expression of bean a-amylase inhibitor in peas results in altered structure and immunogenicity“, J Agricultural and Food Chemistry, 53, 9023-30.), zeigt, das aus gentechnisch veränderten Pflanzen erhebliche gesundheitliche Risiken resultieren. Eine vertiefte Analyse des Falles zeigt (Valenta, R. & Spök, A., 2008, „Immunogenicity of GM peas“, BfN Skripten 239, Bundesamt für Naturschutz, Bonn.), dass die derzeitigen Standarduntersuchungen, wie sie auch von der EFSA vorgesehen sind, in diesem Fall kaum geeignet gewesen wären, dieses Risiko zu erkennen. EFSA muss aus den Fällen wie MON863 und der Amylase-Erbse umfassende Konsequenzen für die eigenen Vorgaben ziehen. Das ist in der jetzt präsentierten Vorlage in keinster Weise erfolgt.
7.2.5. Toxicological testing of the whole GM food/feed	1476-1478 More details are needed on in-vivo testing in systems ''of human origin''. Are human volunteers being considered? Vulnerable groups to possible toxic and allergenicity effects from genetically engineered foods have been identified by the Royal Society and include young babies and people prone to allergic disorders. How is testing on these vulnerable groups proposed?
OTHER SCIENTIFIC COMMENTS	Allgemein Wir möchten an dieser Stelle betonen, das wir hier nur beispielhafte Kritikpunkte vorbringen. Die Punkte dürfen nicht als abgeschlossene Liste verstanden werden. Wir haben uns entschlossen auch zu dem Punkt Monitoring den einen oder anderen Kommentar abzugen, auch wenn dieser Punkt - nach den Vorstellungen der EFSA - nicht Teil des Verfahrens sein soll. Unabhängige Risikoforschung stärken Außerdem sind wir der festen Überzeugung, dass die unabhängige Risikoforschung gestärkt werden muss. Zumindest ein Teil der Risikobewertung von GVO muss von unabhängigen Gutachterinnen und Gutachtern durchgeführt werden. Die EU muss dafür ein plausibeles Konzept vorlegen, in dem die Antragsteller an der Finanzierung der unabhängigen Risikoforschung beteiligt werden, soll heiß, diese in weiten Teilen finanzieren. Monitoring Im draft guidance document wird in den allgemeinen Einlassungen über einen Umwelt-Beobachtungsplan (auch: Umweltverträglichkeitsprüfung - Environmental Monitoring Plan) ein dafür wichtiger Grundsatz aus dem Anhang der Freisetzungs-Richtlinie (2001/18) der EU unterschlagen: Neben den im draft guidance document

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	genannten „direct or indirect, immediate and/or delayed adverse effects of GMOs, their products and their management to human health or the environment, after the GMO has been placed on the market" (direkten oder indirekten, unmittelbaren und/ oder verzögerten negativen Effekten [gentechnisch veränderter Organismen] GVO, ihrer Produkte und deren Management auf die menschliche Gesundheit oder die Umwelt, nachdem die GVO inverkehr gebracht worden sind), besteht der Freisetzungs-Richtlinie zufolge ein „allgemeiner Grundsatz für die Umweltverträglichkeitsprüfung darin, dass eine Analyse der mit der Freisetzung und dem Inverkehrbringen zusammenhängenden ‚kumulativen langfristigen Auswirkungen¿ durchzuführen ist". Die EFSA fordert ein fallspezifisches Monitoring (eine fallspezifische Beobachtung) für einen GVO nur in solchen Fällen, wenn ein etwaiges Risiko bereits annähernd erwiesen ist. („Where there is scientific evidence of a potential adverse effect linked to the genetic modification, then case-specific monitoring should be carried out after placing on the market" - Gibt es eine wissenschaftliche Evidenz für einen potentiellen negativen Effekt, der mit der gentechnischen Veränderung [des GVO] soll nach dem Inverkehrbringen eine fallspezifische Beobachtung durchgeführt werden - draft guidance document line 2212ff) Wir denken, dass dies nicht plausibel ist, da es in der Regel nicht leistbar ist, bereits auf der Basis der Risikobewertung derart umfassend - mit wissenschaftlicher Evidenz - „vorherzusehen" und/ oder zu beschreiben, welcher Art die Risiken sind, die von einem bestimmten GVO für eine bestimmte Umgebung/ für eine bestimmte Umwelt ausgehen. Dies ist umso bedeutender, als dass die zitierte Einschränkung auch im Kontext von möglichen unvorhergesehenen negativen Effekten („Monitoring of effects: Foreseen and unforeseen") beschrieben wird. Ein fallspezifisches Monitoring sollte demgegenüber die konsequente Fortführung des fallspezifischen Zulassungsverfahrens und entsprechend obligatorisch sein. Da das Monitoring ganz wesentlich auf der Grundlage des Vorsorgeprinzips beruht und sich nicht nur auf bereits bekannte und abgesicherte Risiken beschränken darf, ist die Entscheidung über die Art und Weise des Monitorings grundsätzlich dem risk management zuzuordnen. Die EFSA sollte sich darauf beschränken bei allen ihren Stellungnahmen alle möglichen Elemente eines fallspezifischen Monitoring zu beschreiben, unabhängig davon ob sie die Durchführung eines fallspezifischen Monitorings für notwendig hält. Die Entscheidung über das Monitoring muss aber auf der Ebene des risk management getroffen und auch politisch verantwortet werden.
7.2.5. Toxicological testing of the whole GM food/feed	1449-1461 Toxicological testing of the whole plant should always be undertaken, regardless of the extent of the modification. The complex and ill-understood mechanisms by which genes interact with each other make it imperative thata comprehensive evaluation be undertaken on all proposed changes tot he geneic make-up.
6. General recommendations	Crash-Test Wir denken, dass es nach wie vor eine Reihe grundlegender unbeantworteter Fragen zu gentechnisch veränderten Pflanzen (GVP) gibt. Diese weißen Flecken stehen einer wissenschaftlich zuverlässigen Bewertung gentechnisch veränderter Pflanzen im Wege. Das xxxx hat in diesem Zusammenhang am Beispiel des gentechnisch veränderten insektengiftigen Mais MON810 des US-Konzern Monsanto gefordert, gentechnisch veränderte Pflanzen obligatorisch einem Crash-Test zu unterziehen, bei dem bestimmte biotische und abiotische Faktoren und ihre Einflüsse auf den GVO untersucht werden.(www.gen-ethisches-netzwerk.de/gen/2008/crash-test, siehe auch Then, Christoph & Lorch, Antje, 2008 „A simple question in a complex environment: How much Bt toxin do genetically engineered MON810 maize plants actually produce? ", in Breckling, B., Reuter, H. & Verhoeven, R. (2008) Implications of GM-Crop Cultivation at Large

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	Spatial Scales. Theorie in der Ökologie 14. Frankfurt, Peter Lang. (in print)) Transparenz Wir halten es zudem für notwendig, dass die Verfahren an verschiedener Stelle transparenter gestaltet werden: (1) Antragsdossiers müssen weit gehend veröffentlicht werden. (2) Entscheidungswege, Gremienbesetzungen und Entscheidungen der EU-Zulassungen müssen deutlich transparenter werden. Vielmehr müssen alle gentechnisch veränderten Pflanzen per se einem risk assessment unterworfen werden (Siehe UPDATED GUIDANCE DOCUMENT FOR THE RISK ASSESSMENT OF GENETICALLY MODIFIED PLANTS AND DERIVED FOOD AND FEED, The EFSA Journal (2008) 727, 1-135, Draft document adopted in May 2008 , Seite 16: „Where no comparator can be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM crop derived food/feed per se should be carried out."), der von der Methodik auf einer Art Crash-Test (siehe oben) aufgebaut ist. Das heißt die Pflanzen werden unter verschiedenen Bedingungen (zum Beispiel verschiedenen Umweltstressreizen) und auf längere Zeit gezielt auf Schwachstellen (wie Schwankungen in der Expression des neuen Gens) untersucht. Als ein Standard sollten Test unter kontrollierten Bedingungen einer Klimakammer eingesetzt werden (www.gen-ethisches-netzwerk.de/gen/2008/crash-test). Weiterhin müssen potentielle Auswirkungen auf das Nahrungsnetz umfassend und unter voller Berücksichtigung von unerwarteten Effekten bei Ziel- und Nichtzielorganismen, auf allen Stufen des Nahrungsnetzes untersucht werden.
D. INFORMATION RELATING TO THE GM PLANT	Bewertung Herbizid-resistenter GVO mit ihrem Herbizid Trotz der Zuordnung der Risikobewertung von so genannten Pflanzenschutzmitteln (= Unkrautvernichtungsmittel, Herbizide oder andere obligatorisch in Verbindung mit einem GVO ausgebrachte Mittel) in den Zuständigkeitsbereich der Richtlinie 91/414/EEC (... „vom 15. Juli 1991 über das Inverkehrbringen von Pflanzenschutzmitteln" - siehe zum Beispiel draft guidance document line 429ff) halten wir es für unabdingbar, dass eine intensivere Bewertung von einem GVO in Verbindung mit dem (seinem) Herbizid vorgenommen wird. Dies gilt sowohl für die Untersuchung des GVO selbst als auch für die Untersuchung möglicher schädlicher Effekte durch die vermehrte Anwendung des Herbizids in Verbindung mit dem GVO. Es mag sinnvoll sein, die Unkrautvernichtungsmittel auch für sich genommen - entsprechen der Richtlinie 91/414/EEC - zu überprüfen, dem soll nicht per se widersprochen werden.
III. INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED	Antragsdossiers - case by case & step by step Die Europäische Union muss die Antragsteller insofern stärker in die Pflicht nehmen, als dass diese nachvollziehbare, einheitliche, möglichst standardisierte Unterlagen in ihren Dossiers vorlegen, ohne dass dies zu Lasten eines der beiden Grundprinzipien des Zulassungsverfahrens der Europäischen Union geht. Diese Prinzipien sind die Fallspezifität der Zulassungs- bzw. Genehmigungsverfahren (case by case), das heißt jeder gentechnisch veränderte Organismus muss einem eigenständigen Verfahren unterzogen werden. Außerdem müssen einer gegebenenfalls stattfinden Freisetzung eines GVO die schrittweise Entwicklung und Bewertung von der Laborbank über (verschiedene weitere) geschlossene Systeme (Gewächshäuser) vorausgehen (step by step). Dabei ist auf jeder Stufe die Berücksichtigung und Darstellung der Ergebnisse der jeweils vorhergehenden Stufe zu achten.
2.2 Concept of	Familiarity und substantial equivalence Das draft guidance document nennt zwei Konzepte, die im Rahmen von

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substantial equivalence or comparative safety assessment	vergleichenden Überprüfungen von gentechnisch veränderten Organismen (mit ihren isogenen konventionellen und ggf mit ihren Eltern-Linien) zur Anwendung kommen (sollen): Diese sind familiarity (etwa: Vertrautheit oder Bekanntheit) und die substantial equivalence (Substantielle Äquivalenz; etwa: wesentliche Gleichwertigkeit). Unbeschadet der im draft guidance document genannten Bezüge zu den Arbeiten internationaler Organisationen (OECD, 1993a. Safety Considerations for Biotechnology: Scale-up of Crop Plants. OECD, 1993. www.oecd.org/dataoecd/26/26/1958527.pdf?channelId=34537&homeChannelId=33703&fileTitle=Safety+Considerations+for+Biotechnology+Scale-up+of+Crop+Plants; OECD, 1993b. Safety evaluation of foods derived by modern biotechnology: concept and principles. OECD, 1993. www.oecd.org/dataoecd/57/3/1946129.pdf?channelId=34537&homeChannelId=33703&fileTitle= Safety+Evaluation+of+Foods+Derived+by+Modern+Biotechnology+-+Concepts+and+Principles. (der im EFSA draft guidance document angegebene Internet-Link ist nach unseren Erfahrungen nicht aktuell.); WHO/FAO, 2000. www.fao.org/3245 g/es/ESN/food/pdf/gmreport.pdf www.fao.org/es/esn/food/risk_biotech_aspects_en.stm. (die im EFSA draft guidance document angegebenen Internet-Links sind nach unseren Erfahrungen nicht aktuell. NEU: http://www.fao.org/wairdocs/ae584e/ae584e00.htm, abgerufen am 19.09.08)) über die Bewertung der Umwelt- und Nahrungsmittelsicherheit von GVO, muss festgestellt werden, dass die Aussagekraft der genannten Konzepte im Rahmen der Risikobewertung von GVO in einer Reihe von wissenschaftlichen Publikationen kritisiert werden und diese nicht selten zu dem Schluss gekommen sind, dass es unter Anwendung neuerer Erkenntnisse der Genomforschung, in Zweifel gezogen werden muss. Siehe für eine Übersicht zum Beispiel: Terje Traavik, Kaare M. Nielsen und David Quist: „Genetically engineered cells and organisms: substantially equivalent or different?"; in: Terje Traavik und Lim Li Ching (eds.): Biosafety First; 2007. Generell ist weder das Konzept der familarity noch das der substantiellen Äquivalenz der neuen Qualität der wissenschaftlichen Fragen, die sich in der Ära der Postgenomik stellen, angemessen. Die Ergebnisse der Grundlagenforschung zeigen, dass die Wechselwirkungen im Genom wesentlich komplexer sind als ursprünglich angenommen (The Encode Project Consortium, 2007, „Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project", Nature, Vol 447, 14. Juni 2007, Seite 812.; Richard M. Clark, Gabriele Schweikert, Christopher Toomajian, Stephan Ossowski, Georg Zeller, Paul Shinn, Norman Warthmann, Tina T. Hu, Glenn Fu, David A. Hinds, Huaming Chen, Kelly A. Frazer, Daniel H. Huson, Bernhard Schölkopf, Magnus Nordborg, Gunnar Rätsch, Joseph R. Ecker, Detlef Weigel: Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana Science, July 20, 2007. Siehe auch: Presseerklärung der Max Planck Gesellschaft, 20.7. 2007, http://www.mpg.de/english/illustrations Documentation/documentation/pressReleases/2007/pressRelease20070718/index.html.). Da bei gentechnischen Eingriffen nicht nur die genetische Information, sondern auch die Gen-Regulation teilweise außer Kraft gesetzt wird (siehe beispielsweise siehe beispielsweise Diehn, S. et al, 1996, Problems that can limit the expression of foreign genes in plants ..", Genetic Engineering, Vol 18, Seite 83-99.), ist grundsätzlich davon auszugehen, dass die Ähnlichkeit zwischen konventioneller Pflanze und gentechnisch veränderter Pflanze nicht Ausgangspunkt und Basis der Risikobewertung sein kann.
2.1 Concept of familiarity	Familiarity und substantial equivalence Das draft guidance document nennt zwei Konzepte, die im Rahmen von vergleichenden Überprüfungen von gentechnisch veränderten Organismen (mit

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	ihren isogenen konventionellen und ggf mit ihren Eltern-Linien) zur Anwendung kommen (sollen): Diese sind familiarity (etwa: Vertrautheit oder Bekanntheit) und die substantial equivalence (Substantielle Äquivalenz; etwa: wesentliche Gleichwertigkeit). Unbeschadet der im draft guidance document genannten Bezüge zu den Arbeiten internationaler Organisationen (OECD, 1993a. Safety Considerations for Biotechnology: Scale-up of Crop Plants. OECD, 1993. www.oecd.org/dataoecd/26/26/1958527.pdf?channelId=34537&homeChannelId=33703&fileTitle=Safety+Considerations+for+Biotechnology+Scale-up+of+Crop+Plants; OECD, 1993b. Safety evaluation of foods derived by modern biotechnology: concept and principles. OECD, 1993. www.oecd.org/dataoecd/57/3/1946129.pdf?channelId=34537&homeChannelId=33703&fileTitle= Safety+Evaluation+of+Foods+Derived+by+Modern+Biotechnology+-+Concepts+and+Principles. (der im EFSA draft guidance document angegebene Internet-Link ist nach unseren Erfahrungen nicht aktuell.); WHO/FAO, 2000. www.fao.org/3245 g/es/ESN/food/pdf/gmreport.pdf www.fao.org/es/esn/food/risk_biotech_aspects_en.stm. (die im EFSA draft guidance document angegebenen Internet-Links sind nach unseren Erfahrungen nicht aktuell. NEU: http://www.fao.org/wairdocs/ae584e/ae584e00.htm, abgerufen am 19.09.08)) über die Bewertung der Umwelt- und Nahrungsmittelsicherheit von GVO, muss festgestellt werden, dass die Aussagekraft der genannten Konzepte im Rahmen der Risikobewertung von GVO in einer Reihe von wissenschaftlichen Publikationen kritisiert werden und diese nicht selten zu dem Schluss gekommen sind, dass es unter Anwendung neuerer Erkenntnisse der Genomforschung, in Zweifel gezogen werden muss. Siehe für eine Übersicht zum Beispiel: Terje Traavik, Kaare M. Nielsen und David Quist: „Genetically engineered cells and organisms: substantially equivalent or different?"; in: Terje Traavik und Lim Li Ching (eds.): Biosafety First; 2007. Generell ist weder das Konzept der familarity noch das der substantiellen Äquivalenz der neuen Qualität der wissenschaftlichen Fragen, die sich in der Ära der Postgenomik stellen, angemessen. Die Ergebnisse der Grundlagenforschung zeigen, dass die Wechselwirkungen im Genom wesentlich komplexer sind als ursprünglich angenommen (The Encode Project Consortium, 2007, „Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project", Nature, Vol 447, 14. Juni 2007, Seite 812.; Richard M. Clark, Gabriele Schweikert, Christopher Toomajian, Stephan Ossowski, Georg Zeller, Paul Shinn, Norman Warthmann, Tina T. Hu, Glenn Fu, David A. Hinds, Huaming Chen, Kelly A. Frazer, Daniel H. Huson, Bernhard Schölkopf, Magnus Nordborg, Gunnar Rätsch, Joseph R. Ecker, Detlef Weigel: Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana Science, July 20, 2007. Siehe auch: Presseerklärung der Max Planck Gesellschaft, 20.7. 2007, http://www.mpg.de/english/illustrations Documentation/documentation/pressReleases/2007/pressRelease20070718/index.html.). Da bei gentechnischen Eingriffen nicht nur die genetische Information, sondern auch die Gen-Regulation teilweise außer Kraft gesetzt wird (siehe beispielsweise Diehn, S. et al, 1996, Problems that can limit the expression of foreign genes in plants ..", Genetic Engineering, Vol 18, Seite 83-99.), ist grundsätzlich davon auszugehen, dass die Ähnlichkeit zwischen konventioneller Pflanze und gentechnisch veränderter Pflanze nicht Ausgangspunkt und Basis der Risikobewertung sein kann.
MANDATE OF EFSA AND THE GMO PANEL	Wir möchten unserer Stellungnahme vorwegschicken, dass wir das Verfahren der Beteiligung als sehr unglücklich wahrgenommen haben. Einerseits wurde nur eine verhältnismäßig kurze Frist von zwei Monaten eingeräumt, die auch noch genau in

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	die Sommer(-ferien)-zeit fällt. Andererseits wurde der Entwurf nur in englischer Sprache bereitgestellt, was als Zeichen gewertet werden, kann, dass eine breite Beteiligung der europäischen Zivilgesellschaft nicht das primäre Ziel der Europäische Behörde für Lebensmittelsicherheit (EFSA) gewesen sein kann. Genau das sollte aber der Fall sein ... gerade im Bereich der Gentechnologie als einer Risikotechnologie, zu der sich die Bürgerinnen und Bürger der Europäischen Union - wie zu kaum einer andere Technologie - mit sehr großer Skepsis bis offener Ablehnung äußern. Zudem wäre die Bereitstellung einer Gegenüberstellung der alten und der überarbeiteten Version des draft guidance document in Verbinderung mit einer Kommentierung bzw. Begründung für etwaige Änderungen eine zuvorkommende Art der Präsentation gewesen. Die EFSA sollte sich darüber im Klaren sein, dass sie nicht gegen die Bürgerinnen und Bürger und die Zivilgeschaft der Europäischen Union tätig werden muss, sondern in deren Auftrag. Beteilgte EU-Behörden Grundsätzlich sind wir der Ansicht, dass die Aufgaben im Rahmen der Bewertung von gentechnisch veränderten Organismen in der Europäischen Union stärker nach den Fachkompetenzen der Behörden der EU verteilt werden sollten. Entsprechend plädieren wir dafür, dass die EFSA sich auf den Bereich Nahrungs- und Futtermittelsicherheit konzentriert. Ökologische Fragen sollten eher in der Europäischen Umweltagentur (EEA) bearbeitet werden, entsprechend müssen fehlende Kompetenzen dort ggf. aufgebaut werden. Zeitpunkt der Überarbeitung Zudem halten wir den Zeitpunkt der Überarbeitung des guidance document, angesichts der anhaltenden Debatte über das EU-Zulassungsverfahren gentechnisch veränderter Organismen, für äußerst ungünstig gewählt. Es macht nach unserem Dafürhalten wenig Sinn, das guidance document zu überarbeiten, wenn die Rahmenbedingungen für die Zulassung von gentechnisch veränderten Organismen diskutiert und mit an Sicherheit grenzender Wahrscheinlichkeit auch schon bald geändert werden. Dies gilt umsomehr, als dass die Art und Weise des risk assessment, der Risikoabschätzung/ Risikobewertung, wesentlicher Teil dieser Debatten ist. Die EFSA muss gewährleisten, dass die Ergebnisse dieser aktuellen Diskussionen über die Rahmenbedingungen der Zulassung und Bewertung von GVO in das guidance document einfließen.
	Line 234 - 236 We are concerned about these terms of reference, as they imply that EFSA's role is to assist applicants to obtain consents. This represents a significant bias in favour of the GM industry and against the public interest. The safety of the people of the EU depends upon a strictly impartial role for EFSA, and the protection of public health as a priority; for too long EFSA has been perceived as a "facilitation body" for GM approvals, and having watched EFSA at work for a number of years we consider
7.4.1. Nutritional assessment of GM food	1731 Again this is unacceptable There should be NO room for cosy agreements between EFSA and applicants over what might be "substantially" or "compositionally" equivalent to non-GM comparators -- thereby allowing the evasion of testing requirements.
	1516. Take out the word "substantial" here -- it is meaningless, and allows another

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	"escape clause" for an applicant by cosy agreement with EFSA over what is "substantial" and what is not. Again, public safety MUST be the priority at all times.
7.2.5. Toxicological testing of the whole GM food/feed	1519 Interpretation of relevance of toxicity tests The following paragraphs read like an attempt, in advance, to discredit any "independent" animal feeding trial results that might come forward. The attempts to find advance reasons for disregarding negative health effects as "down to other causes" are patronising and slightly ludicrous -- given that it is much more likely that an applicant"s own experiments will be carefully designed to MASK negative health effects. How often does EFSA uncover scientific corruption and insist on repeat feeding trials? Not often we suspect, given the history of cosy working relationships with the likes of Monsanto and Syngenta, and given the clear intent to FACILITATE approvals.
7.2.5. Toxicological testing of the whole GM food/feed	1520. ".....As noted in the EFSA GMO Panel's report on the conduct of animal trials with GM products (Report of the EFSA GMO Panel working group on Animal Feeding Trials, 2008), any effects observed in the animal trials should be evaluated by experts in order to identify relevant effects. The experts' experience will facilitate the interpretation of the observed effects with respect to potential consequences for the health of humans and animals and thus assess their relevance for the safety of food and feed derived from the GM product." Who are "the experts" here? Does EFSA"s GMO Panel consider itself uniquely qualified to rule on animal trials using GM products that might be conducted by independent scientists? That would be arrogant indeed, and insulting to other honest scientists who may undertake trials without the express approval of the GM patent holders. Traditionally EFSA has dismissed animal feeding trials conducted by groups other than the applicants themselves. That should be a source of shame to EFSA and the EC -- and again the dismissal of discovered negative health effects by Pusztai, Ermakova and other scientists is indicative of complacency and bias at the highest level within the regulatory bodies.
7.4.1. Nutritional assessment of GM food	1727 ..... If the GM food has been assessed as compositionally equivalent to the non-GM comparators except for the introduced trait(s) (see Sections 7.1.2) no further studies to demonstrate nutritional equivalence are required, provided that the new trait(s) is not expected to influence the nutritional characteristics of the food. This is totally unacceptable. No applicant should be allowed to evade proper wholefood testing on animals just because there is a cosy agreement between himself and EFSA that the GM variety is "compositionally equivalent" to something else. That is unscientific, and places the public at risk.
7.4.2. Nutritional assessment of GM feed	1757 Once compositional equivalence has been established in GM feeds modified for agronomic traits, nutritional equivalence can be assumed....." Again we totally disagree. This indicates complacency and connivance with the industry which places the public at risk. And we disagree that past studies have failed to add any new information -- from what we have seen, the design and running of these experiments leaves a great deal to be desired, since they are done under the auspices of

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	the GM patent owners by laboratories whose independence can be seriously questioned.
7.2.5. Toxicological testing of the whole GM food/feed	1463. 90-day toxicity studies. Again we are concerned that these will NOT be insisted upon in most cases. With respect to this statement in the EFSA document on Animal Feeding Trials: "The GMO Panel recognizes the difficulties associated with the design of meaningful feeding studies with whole foods....." We disagree totally with this statement. Proper animal feeding studies can be carefully designed and conducted in order to bring forward meaningful results. We suspect that this statement comes from the fact that the results of many animal feeding studies thus far have thrown up very worrying results. This does not mean that the studies are flawed, but that GM feed is having a negative effect upon the health of test animals. That much is obvious to independent scientists and to the general public -- it is grotesque for advisory committees not to recognize it as well. The statement may also have something to do with the fact that GMO patent holders and applicants will not allow scientists involved in independent feeding studies to have access to their reference materials and GM feed; these "difficulties" should be sorted out not by abandoning a requirement for feeding studies, but by forcing the GM corporations to cooperate instead of cynically blocking work that might throw up "inconvenient" results.
7.2.5. Toxicological testing of the whole GM food/feed	1453 etc. Toxicological testing of the whole GM food/feed using animals should be carried out in case the composition of the GM plant is modified substantially, as may be the case with extensive genetic modifications targeted at (i) specific alterations in the metabolism leading to improved characteristics for human or animal nutrition and/or health, or (ii) improved responses to environmental stress conditions, like salt or metal tolerance, or drought resistance." This is a profoundly dangerous statement, given that "substantial equivalence" is presumed by ACNFP, EFSA and other committees to exist between GM plants and their non-GM breeding lines in almost all cases. As we have said, this is a non-scientific concept that should have no place in science-based deliberations, and all references to it should be deleted. We foresee that in the great majority of cases EFSA will simply accept substantial equivalence from the carefully manipulated and selective "molecular, compositional and agronomic date" submitted by applicants. If you want to determine if a foodstuff is harmful to health, then it MUST be fed in whole food form (and not as some amino acid extract from a bacterium) to animals -- and then to people in controlled experiments. All health effects must be properly measured on wholefoods. There is no way out of it. Anything else is designed to pander to the commercial ambitions of the GM corporations -- and will encourage corrupt science. EFSA will knowingly place the public at risk.
7.2.2. Toxicological testing of newly expressed proteins	1420 et seq. "If the applicant considers that a decision on safety can be taken without conducting a repeated dosing study or that other tests are more appropriate, the applicant must state the reasons for this." This clause should be dropped. It an "escape route" by which an applicant can avoid testing simply by agreeing "substantial equivalence" with FSA. Animal feeding tests of the whole food MUST be conducted in all cases.
7.2. Toxicology	1336 etc. "The requirements of toxicological testing must be considered on a case-by-case basis

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	and will be determined by the outcome of the comparative analysis, i.e. the differences identified between the GM product and its conventional comparator....." We object to this clause, as it allows the applicant, with the agreement of EFSA, to simply say that his GM variety is substantially equivalent to its comparator line, thereby avoiding the need for proper toxicological testing. That is for the convenience of the applicant, and places the public at risk. There MUST be toxicological testing -- including animal testing -- of the whole plant for all applications.
7.1.6. Effect of processing	1270 ..... The applicant should provide the scientific rationale for the risk assessment of these products. On a case-by-case basis, experimental data may be required...." There is a long history of corrupt science here, with Monsanto and other companies designing processing / cooking experiments specifically to fit the "no harm" scenario. We trust that EFSA will again insist on whole food tests which replicate the industrial or domestic processing that will occur in the real world. Otherwise such tests are useless.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	1169 et seq. ..... the following paragraph is another extraordinary one, indicative of EFSA"s tortuous and incomprehensible attempts to turn something non-scientific (ie the concept of substantial equivalence) into something pseudo-scientific. Please just get rid of this paragraph.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	1009 etc This is an extraordinary paragraph, explaining how an applicant is supposed to demonstrate that his new GM variety is both unique and "not different" at the same time. This is of course nonsense, with or without the null hypothesis -- and it arises from the obsession with the concept of substantial equivalence. EU law is perfectly clear on the point that GM plants are DIFFERENT from their non-GM isolines -- if they were not different, we would not have all these regulatory procedures in place. Kindly accept that GM varieties are different, and uniquely unstable and unpredictable in their behavior -- and spare us all this convoluted wordage.
4.1.3 Exposure assessment	685 ..... "In this respect specific attention will be paid to that GM food/feed which is aimed at modifying nutritional quality." This is a cynical attempt to reduce surveillance of GM products and crops coming forward for approvals. Since most new GM lines are claimed by their developers to be "nutritionally equivalent" to their non-GM counterparts, it is implied here that these will in effect be deemed to be hazard - free. That again is dangerous, and should NEVER be assumed. This clause is against the public interest.
2.3 Intended and unintended effects	598 etc re unintended effects. We are concerned that EFSA is guiding applicants here into studies (and hence submissions contained in dossiers) which are partial and highly selective -- and which are in effect designed to mask unintended effects. We have no confidence in the scientific integrity of Monsanto, Syngent or any of the other GM corporations who are responsible for the majority of applications. This clause (as written) may be a part of the "grand design" to move away from in vivo studies and into in vitro studies and computer modelling. This is profoundly dangerous -- the only studies which can give sound guidance are WHOLE PLANT STUDIES. Only these will throw up genuinely unintentional and unpredictable effects. Again this clause has the effect of placing the public at risk -- and it is unacceptable as written.
2.2 Concept of substantial	564-584 We object to the continuing use of the "concept of substantial equivalence." This is a non-scientific concept that should have no place in

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equivalence or comparative safety assessment	science-based deliberations, and all references to it should be deleted. We foresee that in the great majority of cases the advisory committees will simply accept substantial equivalence from the carefully manipulated and selective "molecular, compositional and agronomic date" submitted by applicants. Thus the use of the concept becomes a key part of the approvals process. This is against the public interest.
2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL	Line 336 "...applicants have to provide reliable, up to date and comprehensive data." These words should mean what they say. In our view EFSA has been far too lax in accepting partial, heavily manipulated and even corrupt information from applicants for consent -- and it has lost the confidence of NGOs and independent scientists in the process. We also consider that all such data should be placed in the public domain, with none withheld on "commercial in confidence" grounds. Applicants generally have enough protection under patent laws, and the public interest demands full disclosure of everything submitted to EFSA.
OTHER SCIENTIFIC COMMENTS	Line 1907: A general comment is that the document keeps asking for impacts to be described; this is not the purpose of risk assessment, which is intended to determine the probability and magnitude of harm. It is very difficult, and unnecessary, to catalogue all possible changes that may occur following cultivation of the GMO. Line 1909: What is “environmental fitness”? Please clarify. Line 2026: “The selective advantage of any transferred trait should be evaluated in different habitats...”. Please clarify. The endpoint should be operational – increased abundance of the wild relative etc. not a vague term like “fitness”. Line 2056: This paragraph should be removed or updated. Again this is the wrong description of “tiered risk assessment”, it is not the sequence in which data is gathered (see comment on line 623). Line 2637: “the potential impact on target organisms should be assessed in one year field trials initially”. This statement needs revision as its scientific validity is highly questionable. A synergism study in controlled laboratory conditions has far more power to detect potential effects. Line 2644: Same as above. NTO studies are unnecessary if the synergism study shows no effect. Line 2676: See previous comments on the EFSA’s version of tiered testing. Line 2693: Risk assessment should start with what one wants to protect and definitions of harm– it should not start by cataloguing things like cultivation area and routes of exposure. Line 2702: See comment for line 623. Line 2875: Predicting impacts of GMOs on complex ecosystems may be difficult; but that isn’t risk assessment. Risk assessment can be very simple – the GMO is no more harmful than a non-GMO. We don’t have to predict precisely what each does; only that one isn’t different from the other. III.D.11 - Monitoring

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	Line 2306 – 1321: We suggest taking out “In conjunction with human population screening methods currently used by public health organizations (for assessing such elements as incidences of allergic reactions)”. The applicant will collect its information directly from the farmer through farm questionnaires. If a potential unanticipated adverse effect on human health were to occur, it is very likely to be observed by people handling the GMO and thus to be reported in the questionnaire. In case of such observation, the applicant will conduct further analysis of the reported adverse effect to confirm whether it is related to the GMO. III.D.12 - ERA of GM plants containing transformation events combined by conventional breeding Line 2631-2632: ‘the most appropriate comparators”: plural, requires clarification Line 2637-2642: Impact on NTOs should be assessed in one year field trials. The tiered approach should be followed. Line 2644-2652: Impact on NTOs should be assessed in one year field trials. The tiered approach should be followed.
OTHER SCIENTIFIC COMMENTS	Detailed comments: Line 623: The tiered approach here does not seem to offer the possibility of stopping testing at lower tiers should results indicate minimal risk. Tiered testing is not the order in which one collects hazard and exposure data; it is the testing of hypotheses of no harm. Testing starts with lower studies that are most likely to falsify hypotheses that are generally applicable, and, if these hypotheses are falsified, moves to higher tier studies that test more specific hypotheses (e.g., a laboratory study with high concentrations of protein may be applicable to all crops expressing that protein wherever they are grown, whereas field studies may only be applicable to similar environmental conditions). Line 625: Tier 1 is more than hazard identification – it is the comparison of a measure of hazard with a measure of exposure. Tier 1 Hazard Identification does not necessarily mean exposing organisms to the GM plant AND its products. You can identify hazard by exposing organisms to the GM plant OR its products - whichever is most appropriate to provide useful data for the risk assessment (we do not want to have to both protein AND plant material testing but wish to preserve flexibility). Line 630: Tier 2 studies should not necessarily be studies on trophic layers. Tier 2 studies are similar in concept to tier 1, but the risk is estimated under more realistic conditions. Line 636: Tier 3 studies are not exposure studies – they are studies that estimate risk directly without explicitly estimating hazard and exposure. The section should be written so that each tier estimates risk in a particular way (Tier 1 very conservative estimates of hazard and exposure; tier 2 more realistic estimates of hazard and exposure; tier 3 direct measurement of risk etc.). Risk assessments estimate RISK, and it is this that is done in a tiered manner – tiered testing is not the sequential collection of hazard and exposure data. An exception is that it may be possible to estimate risk from exposure only, but only if one can show exposure is negligible such that whatever the hazard, risk is minimal (see comments on Line 658).

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	Line 658: It is too restrictive to say that risk assessments must always begin with hazard identification. It may be possible to demonstrate minimal risk through no exposure. Sections 3 and 4 are very inflexible. They prescribe data that must be collected, without recognising that the data are not the end, but the means to test hypotheses of no harm. There should be flexibility to demonstrate no harm through testing hypotheses with existing data. “Case-by-case” seems to have become “collect the same data for every event”, not a flexible system for testing the most suitable risk hypotheses with the most suitable data. There should be more emphasis on what should be protected – without this is not possible to judge whether the data requirements are the most effective way to estimate risk.
OTHER SCIENTIFIC COMMENTS	Section III.D.9 - D.10 Environmental Risk Assessment General comments: While we understand that the environmental risk assessment guidelines are still under review due to the current self-tasking mandate of EFSA, the text in the present Updated guidance document should be updated so it does not lead to confusion. For example, Section 3 suggests that the risk assessment should comprise studies done at all tiers, whilst Section D.9.5 states that “If first tier tests do not identify sensitivity in exposed species then second and third tier tests may not be required” and Section 12 proposes a minimum of one year field testing, eventually followed by further laboratory tests, where appropriate. While the text in Section D.9.5 is consistent with internationally recognized approaches to tiered risk assessment, the texts in Sections 3 and 12 highlight that there is confusion on what a tiered risk assessment is, compared with a step-by-step data gathering exercise. The step-by-step risk assessment refers to gathering data to assess risk (on hazard and exposure), while the tiered approach refers to an approach that measures risk at each step and allows risk assessors to establish whether or not more data are needed to make an assessment. If more data are considered necessary, then these are generated, either on hazard or on exposure or both, using more refined methods than in previous tiers. The confusion should be removed to allow the applicant to develop a logical risk assessment package, based on a tiered approach. Field trials are only useful if they can be designed to test a clear hypothesis. Field testing should not be a requirement unless lower tier studies indicate the need for this. If additional studies at higher tiers are requested, their purpose should be made clear so they can be designed to respond a specific question to facilitate a regulatory decision, not as a blanket request for detecting potential “unknowns”. More effort on providing clarity on risk assessment endpoints is needed. Overall Sections D.8 and D.9 do not appear to have changed considerably from the approach suggested in the previous guidance. This is disappointing since there have been many proposals and advances in the definition of tiered risk assessment approaches and many publications that could have been incorporated to produce a more up-to-date guidance. In section D.12 there are some points referring to the environmental risk assessment for stacks that XXX and XXX have already made to EFSA. For

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	example, the request for one year of field trials “initially” with target and non-target organisms to assess potential interactions between proteins. These data requirements are clearly in conflict with the tiered risk assessment approach and should be reviewed.
V. REFERENCES	References Lines 3161-3162: The web address is not correct. It should be read “ http://www.oecd.org/document/9/0,3343,en_2649_201185_1812041_1_1_1_1,00.html” Recommended references to be added into the reference section Codex Alimentarius Commission (CAC)(2003), Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June-5 July, 2003. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity. 47-60. Constable, A., Jonas, D., Cockburn, A., Davi, A., Edwards, G., Hepburn, P., Herouet-Guicheney, C., Knowles, M., Moseley, B., Oberdörfer R. 2007. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chemical Tox. 45:2513-2525. Delaney, B., Astwood, J. D., Cuny, H., Eichen Conn, R., Herouet-Guicheney, C., MacIntosh, S., Meyer, L. S., Privalle, L. Goa, Y., Mattsson, J., Levine, M. (2008) Evaluation of protein safety in the context of agricultural biotechnology. Food and Chem. Toxicology 46: S71-S97. Goodman, R.E. (2006). Practical and predictive bioinformatics methods for the identification of potentially cross-reactive protein matches. Mol Nutr Food Res 50:655-660. Goodman, R.E. ,Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R. (2008). Allergenicity assessment of genetically modified crops—what makes sense? 26:73-81. Hileman, R.E., Silvanovich, A., Goodman, R.E., Rice, E.A., Holleschak, G., Astwood, J.D. and Hefle, S.L. (2002). Bioinformatic methods of allergenicity assessment using a comprehensive allergen database. Int. Arch. Allergy Immunol. 128:280-291 Ladics, G.S., Bannon, G.A., Silvanovich, A., and Cressman, R.F. (2007). Comparison of conventional FASTA identity searches with the 80 amino acid sliding window FASTA search for the elucidation of potential identities to known allergens. Mol. Nutr. Food Res. 51:985-998. McClain, S., Vieths, S. and Bannon, G. A. (2008) Assessment of Food proteins for Allergenic Potential in Food Safety of Proteins in Agricultural Biotechnology (Hammond, B. G., ed), CRC Press (Boca Raton, FL). Thomas, K., Bannon, G., Hefle, S., Herouet, C., Holsapple, M., Ladics, G., MacIntosh, S., Privalle L. (2005). In silico methods for evaluating human allergenicity of novel proteins: International Bioinformatics Workshop Meeting Report: Mallorca, Spain, February 23-24. Tox Sciences 88:307-310 Thomas, K., Gendel, S. M., Herouet-Guicheney, C., Ladics, G., McClain, S.,

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3. ISSUES TO BE CONSIDERED FOR RISK CHARACTERISATION	Lines 2842-2843: Given the diversity of processing methods, guidance is requested.
OTHER SCIENTIFIC COMMENTS	Line 1980: “Section III, D 9.5.”, should read “Section III, D 9.3.”.
7.7. Post-market monitoring of GM food/feed	Lines 1887-1891: While it is stated that practically, PMM studies are difficult to perform, the draft guidance does list some specific cases where it should be required (e.g., GM functional foods). Given these GM functional foods will be produced using commodity crops such as corn or soybean which are contained in numerous processed foods and that the human diet is so diverse, it is unlikely that such studies will yield any useful information in regard to the safety of the product. References Codex Alimentarius Commission (2003). Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants and Appendix IV, Annex on the assessment of possible allergenicity, 25th Session, Rome, Italy 30 June–5 July, 2003. pp. Goodman RE, Vieths S, Sampson HA, Hill D, Ebisawa M, Taylor SL, van Ree R. (2008) Allergenicity assessment of genetically modified crops--what makes sense? Nat Biotechnol. 26(2):241. Hileman, R. E., Silvanovich, A., Goodman, R. E., Rice, E. A., et al.(2002) Bioinformatic Methods for Allergenicity Assessment Using a Comprehensive Allergen Database. Int. Arch. Allergy Immunol. 2002, 128, 280-291; Stadler, M. B. Stadler, B. M., Allergenicity prediction by protein sequence. FASEB Journal 2003, 1141-1143. Jenkins, J.A. et al. Evolutionary distance from human homologs refelcts allergenicity of animal food proteins, J. Allergy Clin Immunol, 120:1399-1405, 2007 Ladics, G.S., Bannon, G.A., Silvanovich, A., and Cressman, R.F. (2007). Comparison of conventional FASTA identity searches with the 80 amino acid sliding window FASTA search for the elucidation of potential identities to known allergens. Mol. Nutr. Food Res. 51(8):985-998. McClain, S. and Bannon, G.A. (2006) Animal Models of Food Allergy – Opportunities and Barriers. Current Allergy and Asthma Reports, 6: 141-144.

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7.6. Conclusion of the toxicological/nutritional and allergenicity assessment	Lines 1856 – 1857: In absence of comparator, it is not scientifically sound to accept that the allergenicity assessment should conclude on the likelihood of allergenicity of the novel GM food. A few foods are major allergenic foods and the majority is not allergenic for the whole population. Lines 1858 – 1860: This statement should be clarified as there is no evidence to modify allergenicity by additive, synergistic or antagonistic effects. More generally, to define these adverbs could help the scientists to understand the question.
7.5. Anticipated intake/extent of use	Line 1805: It would be extremely useful for the EFSA guidance to reference sources (e.g. databases) of consumption data that are publicly available.
7.4.1. Nutritional assessment of GM food	Line 1727 – 1730: This statement implies that a 90-day rat feeding study is not required if the food is compositionally equivalent to the comparators except for the trait. If this is correct, it is suggested to state it unequivocally.
7.3.2. Assessment of allergenicity of the whole GM plant or crop	Lines 1679 to 1681: If there are no differences between the protein profiles (on 2D-gels), there is no need to confirm it by using human or animal serum or other functional assays. And more generally, there is still no substantial proof that profiling techniques (proteomics, metabolomics, transcriptomics) have been developed to the point where scientists can easily interpret differences that are likely related to the gene insertion or the activity of the inserted DNA, RNA or protein in the context of safety. Such comparisons are complex. The transgenic organism rarely (if ever in the complex organisms developed to date) has an isogenic comparator. The host is rarely (if ever) homozygous before transformation. Once transformation is made and lead lines are selected they are often crossed into different elite lines and then backcrossed to obtain inbred lines that have many genetic differences from the transformed line. For crops, environmental factors (temperature, moisture, light, wind, soil conditions, pests, etc.) modulate the expression of many genes through transcriptional and post-transcriptional modification so that the resulting proteome, metabolome or transcriptome between any two genetically identical individual plants will vary. Individuals with some additional genetic differences (similar varieties of soy, corn, wheat), can vary significantly more. How much difference is too much for human safety and which factors are likely to be meaningful in terms of safety? How can scientists rationally set boundaries for factors that they don't

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	know the impact of, or the relative variance in populations and varieties of currently grown similar crops? Lines 1682 – 1687: The extraction of proteins from pollen is very challenging and it is very unlikely that testing pollen proteins is feasible. Further it is unclear how potential respiratory allergy prevalence would be related to supporting the safety assessment of newly expressed proteins in food crops. It should be noted that respiratory allergens are contained in the allergen FARRP database (allergenonline.com) and are routinely searched for any similarity to transgenic proteins in GM crops. More generally, all of the crops that are being transformed have some risk of occupational allergy. Occupational allergy should be managed through safety precautions in the workplace (masks, clothing, gloves, exhaust fans, etc.). What specifically are the risks of respiratory allergy, how should they be evaluated and how much difference is too much? A few GM plants do shed a lot of pollen. The pollen of almost every plant can be allergenic, and there is a huge range of impact/risk. For instance ragweed (Ambrosia sp.) is a major airborne allergen in the U.S., and there are a few allergenic proteins in the pollen. The public health impact is significant (rhinitis, some asthma, etc.). Goldenrod (Solidago altissima) is another member of the sunflower family and has been pointed to as an important allergenic source, yet there is little pollen produced, it is not wind-borne and although there are IgE-cross-reactive proteins (compared to ragweed), there are probably no health consequences due to pollen of this plant. Why would it be important to study the potential impact on respiratory allergy for goldenrod? The same can be said of soybean, potato, rice, cotton and wheat (self-pollinated or requiring chemicals to open the flowers). What is the other risk of airway allergy?
7.3.1. Assessment of allergenicity of the newly expressed protein	Lines 1653-1656: The guidance document indicates that targeted serum screening should be performed. Targeted serum screening is not likely to add value. Cross-reactive IgE is not always predictive of clinical allergy (Vieths, 2002). Codex (2003) does not currently recommend a targeted sera screen study. Rather, the Codex document indicates that "As scientific knowledge and technology evolves, other methods may be considered..." but they must be "scientifically sound". A targeted sera screen does not meet these criteria, as it has not been validated and its utility in predicting protein allergenicity has not been determined (Thomas et al.,2007a). Therefore, we recommend that it be removed from the document. Lines 1657 through 1671: It should be clarified that still in 2008, there are not clear alternative models that have been demonstrated to be even modestly predictive for identifying human food allergens, and validated to the point where they can substitute for specific human serum tests. Some, like basophil/mast cell activation or histamine release can and should be used to supplement IgE binding results that are ambiguous, they are not of use unless there is IgE binding. Others, such as T cell stimulation or TCR/epitope prediction, have not been shown to be predictive for allergy. No animal model has been shown to be predictive and provide a substantial ability to rank allergenic and non-allergenic proteins. While a number of publications and authors have claimed results demonstrating that a given T-cell prediction tool or assay, a structural motif (or overall structural predictor), or a cell based or non-human animal based assay is predictive, and also shown some positive results, the data are lacking to demonstrate that any of these are widely tested and predictive. Such assays need to move beyond testing only ovalbumin, a few peanut allergens and milk allergens. They need to include weak and/or non-allergenic proteins and the overall ranking must be substantial (probably much greater than 70% predictive) before they should be considered as

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	primary tests. The use of lesser predictive tests would need some back-up verification assays that are also highly predictive, otherwise such tests simply add greater uncertainties for predicting relatively rare events (most proteins are not substantial allergens). "The use of existing models…" This reads as if there are models that may be already available. This should be clarified so that it is clear that there are no validated alternative models available. There are numerous references available that state this point very clearly (McClain and Bannon, 2006; Goodman et al., 2008). Currently no validated animal model exists that could be recommended to predict allergenicity. Because of this, we suggest a change to the statement "Animal models are certainly also useful..." to "Animal models may be useful…once validated."
7.3.1. Assessment of allergenicity of the newly expressed protein	Lines 1651 to 1652: It is not clear how it would be useful to perform serum IgE binding comparison with intact, pepsin digested and heat denatured proteins UNLESS the intact protein had some degree of IgE binding and one wanted to evaluate the possible reduction in IgE reactivity that might occur through digestion or processing. There are only one or two clear cases where IgE binding has been markedly increased (or developed) as a result of digestion or processing. If there wasn't any binding, who would be the likely people at risk? Whose sera should be tested? What is the scientific justification? It should be clarified that pepsin resistance is itself just one of the panel of assessments used, as referenced for the "cumulative body of evidence…" line 1594. Additional fragment analysis would only be conducted upon other supporting evidence to suggest potential allergenicity. Small fragments in and of themselves are not indicative or predictive of potential allergenicity. If bioinformatics indicate no concern, then it is equally unlikely that any smaller fragment is similar to an allergen. Full length bioinformatic analysis accounts for any portions of the protein that may have similarity to allergens. Several thousand possibilities exist in order to denature by heat the proteins (wet, dry conditions, several temperatures, heating procedures, etc) as described in Thomas et al paper (2007a). It is not clear why one would conduct this additional IgE screening if the initial IgE sera screening study to confirm or exlude IgE cross-reactivity with the newly expressed protein is negative. We recommend that it be removed from the document.
7.3.1. Assessment of allergenicity of the newly expressed protein	Lines 1651 to 1652: It is not clear how it would be useful to perform serum IgE binding comparison with intact, pepsin digested and heat denatured proteins UNLESS the intact protein had some degree of IgE binding and one wanted to evaluate the possible reduction in IgE reactivity that might occur through digestion or processing. There are only one or two clear cases where IgE binding has been markedly increased (or developed) as a result of digestion or processing. If there wasn't any binding, who would be the likely people at risk? Whose sera should be tested? What is the scientific justification? It should be clarified that pepsin resistance is itself just one of the panel of assessments used, as referenced for the "cumulative body of evidence…" line 1594. Additional fragment analysis would only be conducted upon other supporting evidence to suggest potential allergenicity. Small fragments in and of themselves are not indicative or predictive of potential allergenicity. If bioinformatics indicate no concern, then it is equally unlikely that any smaller

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7.3.1. Assessment of allergenicity of the newly expressed protein	Lines 1621-1626: The FARRP AllergenOnline Protein Allergen Database is a publicly available database (allergenOnline.com) and the first of its kind to be curated and fully peer-reviewed by an international panel of allergy experts. While EFSA appear to be encouraging harmonization, it is noteworthy that all of the major applicants for GM food/feed crop approvals are using this database. It is unclear to applicants if EFSA makes use of different allergen databases and algorithms. If yes, what is the basis for using something different than the FARRP database? Line 1626: reference to proteins belonging to protein families which include a high proportion of allergens seems to imply that a protein belonging to one of these families represents some increased allergenicity risk (even though this is not stated directly). While it is true that plant food, pollen and animal food allergens actually belong to only a small number of protein superfamilies, it is absolutely incorrect to imply that a protein which belongs to one of these families has inherent increased risk of being allergenic. Jenkins, J.A. et al. (2007) demonstrate that allergenicity of protein family members decreases as a function of relatedness to human homologs and, in fact, certain protein families within superfamilies do not contain any known allergens (Radauer, 2007). EFSA is advised to revise the language of this section to avoid subtle implication that belonging to a certain protein family, in and of itself, is informative of allergenic potential. Three dimensional structure analysis of many of the known allergens remains to be developed. There are very few allergens that have the extensive body of data necessary to determine their tertiary structure (Thomas et al., 2005).
7.3.1. Assessment of allergenicity of the newly expressed protein	Line 1608: There appears to be a possible "disconnect" between statements regarding allergenicity to animals on lines 1608 and 1688-1689. In the context of allergenicity of novel proteins, line 1608 specifies that, for stacks, "an assessment of any potential for increased allergenicity to humans and animals should be provided", whereas lines 1688-1689 (in the context of the whole plant) state "Regarding animal health, allergenicity is not a significant issue that needs to be specifically addressed." Lines 1611-1626: There have been a number of publications since the 2001 FAO/WHO and 2003 Codex documents that demonstrate the lack of predictive value for short-amino acid identity matches (6 through 8mer or beyond). As discussed in review articles (e.g. Goodman et al., Nature Biotechnology 2008, 26(1):73-81), the short 100% identity match algorithms do not appear to have a positive predictive value while the longer FASTA/BLAST matches (e.g. >35% identity over 80 or more amino acids) do, although those criteria (35% identity) may be a bit lower than necessary and thus can have a higher than desired false positive predictive value. Importantly the EFSA document does not list the 35% identity match which is the primary matching criteria listed by Codex (2003). That should be corrected as it is one of the two primary reasons for performing specific serum tests. Further, to date no one has developed a three-dimensional

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	comparison algorithm or databases that have been shown to be predictive for allergenic cross-reactivity or for estimating the likelihood that a protein will be an allergen (with any objective criteria for judgment). Line 1615: states “a search for contiguous identical or chemically similar amino acid residues” is required.” Neither Codex nor the 2001 FAO/WHO expert consultation mention “chemically similar amino acid residues”, but only identical residues. Furthermore, multiple recent publications present evidence as to why this type of homology search has no value. Current thinking is that the 35% IDENTITY over 80 or greater amino acids is more meaningful as well as highly conservative (Hileman et al. 2002, Codex 2003, Thomas et al. 2005, Ladics et al., 2007, 2006, Goodman et al., 2008), The use of “chemically similar” amino acid residues would produce a large number of false positive results that would further limit the utility of the assay. The reference to “false negative and false positive” results should at a minimum, reference Silvanovich et al, 2006, for describing the use of a scientifically justified minimum epitope-length search strategy. Suggest rewriting the footnote #10 to say that “Both, a high level of false positives and a high level of false negatives reduce the utility of a small contiguous polypeptide search strategy. In regard to the use of 6 or 7 identical amino acid segment searches, there is a large amount of data and scientifically justifed rationale in the published literature (see refs. below) that indicates the use of < 8 (i.e., 6 or 7) contiguous identical amino acid searches leads to a high level of false positives and therefore, does not contribute to the safety assessment of novel proteins. Based on these data, the use of 8 or greater contiguous identical amino acids should only be advocated for identification of potential IgE epitopes. These relevant references include: Hileman et al., 2002; Stadler et al., 2003; Thomas et al., 2005; In silico methods for evaluating human allergenicity of novel proteins: International Bioinformatics Workshop Meeting , 2006; Silvanovich et al., 2006. The value of short amino acid sequence matches for prediction of protein allergencity. Toxicol. Sci. 90:252-258.
7.3.1. Assessment of allergenicity of the newly expressed protein	Line 1594: The document states that a cumulative body of evidence is necessary to minimize any uncertainty with regard to potential allergenicity and then implies that there is a linearity or decision tree associated with this assessment. Multiple recent publications imply that this is not the case – a weight-of-evidence approach is not a decision tree approach (Codex, 2003; Goodman, 2008; McClain et al., 2008). Line 1602: Additional clarification is requested on how this should be conducted, as currently there are no widely evaluated or validated models either in vitro or in vivo for predicting gluten-sensitive enteropathy. Lines 1607-1610: “Where events have been stacked by conventional crossing an assessment of any potential for increased allergenicity to humans and animals should be provided. These potential effects may arise from additive, synergistic or antagonistic effects of the gene products. This assessment will clearly require a case-by-case approach.” The specific focus of the added paragraph is not clear. Is this paragraph describing potential changes in endogenous allergenicity or simply of the added traits? Is there any evidence that suggests such effects can occur, and if so what types of changes/traits would be evaluated? Are there examples in naturally occurring foods where we know that two relatively non-allergenic proteins behave differently

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	when separate, but behave as additive allergens (?), act synergistically or antagonistically when put together? The same should be asked about allergenic proteins. Assessing possible additive effects of allergenic proteins makes sense. If you had a situation where only allergen 1 is in a plant, allergen 2 in another, and then you add allergen 1 and allergen 2 into the same plant you would expect that some individuals to react more strongly than if they consumed (or were exposed to only one). However, that would not be different than if they ate both the allergen 1 plant and the allergen 2 plant at the same time. There are no cases that we are aware of where there is evidence that putting two proteins together caused synergy. Finally, if two proteins acted antagonistically (as allergens?) that might be a good thing as our interpretation of antagonism would be reduction of the allergenicity of one of the components relative to single events. As it stands, that paragraph and the concepts do not seem to make much sense. If either of the events showed substantial risk of allergy, it would not likely be allowed by regulators. Stacking by conventional breeding within a single crop should not require specific recommendations for additional allergenicity testing if the singles have been shown to pose no allergenic risk. One interpretation of this statement is that additional IgE binding assays may be required for the stack to prove that there was no evidence of increased allergenicity. The rationale behind this statement needs to be further clarified.
7.3. Allergenicity	Line 1578: Please note that allergies affect individuals who have a genetic predisposition and who are also exposed to an allergenic protein. No validated tests, which can permit to assess the sensitization potential of a protein, are available. Only the elicitation phase of allergy could be studied. Lines 1578 and 1584: Food and respiratory allergies are two different conditions. They should not be mixed up. Pollen allergy should be part of the risk management (occupational issue) as it is not possible to test it pro-actively (no validated method for testing pollen allergy). While some consideration should be given to high level expression of the new protein in pollen of plants that produce high amounts of airborne pollen, there are only a few allergenic proteins that seem to have this route of exposure as a significant sensitizing route (profilins, Bet v 1, lipid transfer protein) and that have some connection to risk in food safety (the point of these regulations). Therefore it is suggested to keep the safety assessment on focus for food safety. Lines 1584–1588: The list of all plant allergens is not fully known. In addition, it is impossible to evaluate the over-expression of the natural endogenous allergens as the biological variations of known plant allergens (under different growing conditions) have never been studied. Moreover, little is known about the prevalence of allergies in the EU, which makes this evaluation difficult. In particular, the soybean and mustard are considered to be major allergenic foods in the EU despite the absence of published clinical and epidemiological data that support it. Based on the recent results presented by the European project EUROPREVALL during the recent EAACI 2008 meeting (June 2008), it seems that the prevalence of soybean allergy is extremely low compared to tomato, which does not belong to the big 12 list for instance.
7.3. Allergenicity	Allergenicity While this section in the updated guidance document has not been changed from the 2006 document, we strongly recommend a revision in order to keep the risk assessment and the respective requirements in line with scientific progress and international harmonisation.

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7.2.5. Toxicological testing of the whole GM food/feed	Lines 1556–1569: These 2 paragraphs are a repetition of lines 1476-1478. Lines 1570–1572: The correlation between the adverse effect(s) and the exposure to GM food/feed should be demonstrated first. References Alouf, J. and Freer, J. The Comprehensive Sourcebook of Bacterial Protein Toxins, Second edition, Academic press, New York, 1999. Delaney, B., J.D. Astwood, H. Cunny, R.E. Conn, C. Herouet-Guicheney, S. MacIntosh, L. Meyer, L. Privalle, Y. Gao, J. Mattson, and M. Levine (2008). ILSI International Food biotechnology Committee Task Force on Protein Safety Evaluation of protein safety in the context of agricultural biotechnology. Food Chem. Toxicol. 46:S71-S97. EFSA (2008). Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem. Toxicol. 46: Suppl 1:S2-70. Hammond, B. and A. Cockburn (2008). The safety assessment of proteins introduced into crops developed through agricultural biotechnology; a consolidated approach to meet current and future needs. in Agricultural Biotechnology, B. Hammond (Edit.) CRC Press, Taylor and Francis Group, Boca Raton, pp. 259-288. Pariza, M.W., and E.A. Johnson (2001). Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. Regul. Toxicol. Pharmacol. 33:173-186. WIL Research Laboratory Historical Data Base, 2007 (personal communication).
7.2.5. Toxicological testing of the whole GM food/feed	Lines 1535–1537: In general, it is very difficult to have a dose-response relationship as the high dose selected in the study design is often the highest dose that could be administered to animals (without inducing pleiotropic effects). This highest dose without adverse effects represents the NOAEL. Lower doses are not informative. The approach that is used for testing chemicals is not directly transposable to the case of GM food/feed. Dose response relationships may be clinically relevant in some situations, but not all. For example, there may be a large variability in a certain blood chemistry parameter (eg, alkaline phosphatase in young male rats – range 40 to 140 U/L; mean + 2 SD) (WIL, 2007). There could be higher levels in the high dose animals (eg 70 U/L) compared to the lower dose (eg. 40 U/L) and control (50 U/L), which is dose related yet well within normal variability. If this scenario occurred, and there is an absence of related clinical changes (no increases in other serum liver enzymes, changes in liver weight or liver histopathology), then the biological relevance of the "dose related" changes may be questioned. Some rodent feeding studies have been conducted where only one dose has been employed. These have been accepted by EFSA since the dietary incorporation rate was sufficiently high, but not at levels that would interfere with the nutritional quality of the diets. In this situation, it is not possible to examine dose response relationships, but if there are no biological meaningful differences between the test and control animals in the study, then the comparability of the GM to control variety has been confirmed. EFSA has encouraged registrants to adapt chemical toxicology testing paradigms to the safety evaluation of GM crops. These studies have generally employed more than one dose level, although limit doses studies have also been used and accepted. However, for chemicals, it is possible to test at much higher

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	exposure levels (1000X) than humans would normally encounter, so that if an effect is observed at higher doses, but not at lower doses, and if there is still a sufficient safety margin, the chemical may be approved for use (food additive, pesticide etc). However for GM crops, while acceptable safety margins can be achieved (100X or greater), the regulatory climate is such that if effects would be detected at the highest dose, but not at a lower dose (30X), it is unlikely that the GM crop could still be approved. Thus, there is not any incentive to test GM crops at lower dietary levels. But, if the registrant is willing to test the crop at one higher dietary incorporation rate (eg. ~ 33% corn grain in the diet), and the study confirms there are no adverse effects, then the crop should be considered to be safe for human consumption. In regard to gender specific differences, a case can be made if it is hormonally related (gonad weights) etc, but if it is not directly sex related (changes in serum liver enzymes, BUN, or electrolytes in one sex and not the other) then in the absence of other correlative changes, the biological relevance of such changes in only one sex may be questioned.
7.2.5. Toxicological testing of the whole GM food/feed	Lines 1485–1489: The statement: "Generally, feeding trials with this type of GM foods/feeds is requested in order to assess the impact of consumption on human and animal health. On a case-by-case basis this is also applicable to foods and feeds derived from GM plants obtained through conventional breeding of parental GM lines (stacked events)." should be clarified by using a scientific rationale. What should trigger such a study with combined traits compared to stacked events?
7.2.5. Toxicological testing of the whole GM food/feed	Lines 1463: The scientific criteria that trigger a 90-day study with GM food/feed should be clarified. Lines 1476-1489: EFSA raises a number of potential safety concerns, and then proposes a variety of studies to test for these potential concerns. Unfortunately, they do not provide any perspective regarding the probability these effects can occur, nor on the predictive value of the tests they propose. For example, gene expression profiling whether conducted in vitro or in vivo has not be validated as a predictive model at this time, and EFSA has recently cautioned about the utility of data generated from non- validated tests (EFSA, 2008). In regard to synergistic actions, it is not clear whether EFSA is referring to potential interactions in the plant, in the animal consuming the plant, or both. Many of these hypothetical safety concerns need to be put in the context of conventional plant breeding. Many thousands of genes are exchanged in plant breeding and protein expression and interactions will change in the progeny of parental lines. Despite all of these changes that occur naturally, these interactions very seldom result in adverse changes affecting the safety of the plant for consumption In biotechnology, only a handful of genes or less are introduced, and the prospect that these genes may somehow lead to interactions that could result in detrimental changes is quite remote based on years of experience. The EFSA statement “information on the response to combined administration of proteins to target organisms and regarding effects on the activity of target enzymes” is unclear and needs clarification. When multiple Cry insect control proteins are introduced into a plant, target insects will be fed the combination of Cry proteins to check for evidence of synergistic effects. Usually synergy is not seen as there can be differences in receptor binding to different Cry proteins across species. If there are effects, experience has shown they are generally additive. If there is no evidence of synergy for protein combinations, then the safety of each protein can be assessed individually. For non-target organisms such as mammals, there would be no synergy since they lack high affinity binding receptors for Cry proteins. Early toxicology tests on Bt microbial pesticides that contain mixtures of different Cry

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	proteins (depending on the subspecies; Betz et al., 2000) found no evidence of adverse effects in mammals etc, similar to the absence of adverse effects when one Cry protein is tested individually. In regard to the EFSA statement “activity of target enzymes”, many hundreds of studies have been carried out over the years showing that enzymes used in food production caused no treatment-related effects when fed to laboratory animals (Hammond and Cockburn, 2008). If several enzymes were introduced into a plant to change the metabolism of a pathway to achieve an intended technical effect, it is highly unlikely the enzymes would cause hypothetical synergistic adverse effects if consumed as they would be degraded in the GI tract. Their potential digestibility would have already been confirmed in vitro. Humans consume a large variety of enzymes present in the foods they consume every day without any concerns about potential interactions. EFSA has also raised the possibility that animal feeding studies may be needed with crops containing introduced traits derived from parental lines through conventional breeding. This suggestion is based on hypothetical concerns about potential interaction of traits combined through conventional breeding. It is difficult to understand why a combined trait variety would need to be tested, if the individual parent lines bearing the introduced traits were already tested and shown to be safe. Conventional breeding has been accepted as safe; just because it is used to combine introduced traits from parent lines does not now make conventional breeding unsafe.
7.2.3. Testing of new constituents other than proteins	Lines 1423-1435: Some definition needs to be provided regarding “new constituent”. For example, by introducing an enzyme(s) into a vegetable oil crop, it is possible to modify existing metabolic pathways to produce fatty acids that are not normally present in the oil. The fatty acid would be “new” to the crop, but could also be covered under section 7.2.4 if the fatty acid is found in other food crops (natural constituent) and therefore has a history of consumption. The safety of the altered oil would still need to be assessed, but this situation is not analogous to a new food additive safety assessment. As pointed out, the safety assessment of “new constituents” should be case by case, even though the rest of 7.2.3 deals with listing the many toxicology studies that may be required for assessing the safety of food additives. It would be helpful instead of listing all of the possible toxicology studies that could be needed, to put into perspective whether and when these tests would be needed. Table 2, provides a list of genotoxicity tests for which OECD guidelines exist. This stand-alone list doesn"t seem to serve any useful purpose. No such list is provided for other toxicology endpoints, e.g., carcinogenicity, reproductive or developmental toxicity. Moreover, some of the genotoxicity tests listed are rarely used these days in standard test batteries for chemicals, and many of them are redundant to other tests on the list. It would be more useful to provide examples of the types of products that might trigger additional toxicity tests, and the rationale for triggering specific tests.
7.2.2. Toxicological testing of newly expressed proteins	Lines 1410–1421: the guidance states that repeated dose toxicity studies are not necessary if reliable information regarding the safety of the newly expressed protein (including its mode of action) is available and that the protein is not structurally and functionally related to proteins which have the potential to adversely affect human or animal health (described in lines 1395-1399). In contrast, lines 1415 -1418 say that a 28-day repeated dose oral toxicity study should normally be performed. This implies that the study is done automatically without a trigger suggesting the need for it. Furthermore, lines 1419 – 1421 again state that the applicant must state the reasons if he considers not doing a repeated dose study. This is very contradictory and it would be recommend to reword the paragraph including that the 28-day repeat dose study is done only if it is triggered

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	(Delany et al., 2008). Furthermore, there is inconsistency with the guidance provided by EFSA in a recent publication (EFSA, 2008): " It is emphasized that the above mentioned tests, in essence developed for the safety assessment of chemicals, should only be applied for newly expressed constituents in GM plants and derived food and feed according to need, that is selectively and on a case-by-case basis, depending on the class, novelty and type of substance, data available on structural relationships and toxicity, occurrence and history of use." In addition, the guidance indicates (line 1418) "Depending on the outcome of the 28-day toxicity study … targeted investigations may be required, including analysis of immunotoxicity." Again, it would be helpful if EFSA could provide some example of what kind of results from a 28-day study would trigger immunotoxicity assessment.
7.2.2. Toxicological testing of newly expressed proteins	Lines 1389-1394: The document discusses the importance of biochemical studies to demonstrate the comparability of proteins used in toxicology studies to demonstrate that they are representative of those expressed in planta including enzymatic activity. Obviously, this could present a challenge since the document provides no guidance as to what would be considered an acceptable reason for why they would not be required. It is more troubling that the document does not present any alternatives for situations where it will not be possible to produce and isolate the proteins that are to be tested. Lines 1391- 1394: Suggest adding that thorough enzyme characterization be completed in vitro on the microorganism produced enzyme to be consistent with lines 1379 through 1380 representing the challenges of obtaining sufficient enzyme/protein from the plant material. This could also be added to line 1388 that much of the listed items under the bulleted text are completed on the microorganism produced protein. Line 1400: According to the guidance document, the stability of the protein should be tested in conditions mimicking the normal processing/storage of the plant. As there may exist more than several thousand sorts of processing/storage procedures, we should have some guidance here as this is too vague. Clarification is required on how to characterize the denaturation of proteins and the protein fragments using microorganism produced protein (functional assay, sequencing, SDS-PAGE, western blot, 2D gel, etc). Lines 1403 and 1407: It would be helpful to have a definition of "stable" in the context of "stable protein fragment" and "stable breakdown product". A fragment or breakdown product might degrade over time - Is it considered stable if it shows no change or breakdown for 30 min? 60 min?
7.2.2. Toxicological testing of newly expressed proteins	Lines 1376-1377: The guidance document states that for proteins that have a history of safe consumption by humans and animals, specific toxicity testing may not be required. We would appreciate a reference or definition for "history of safe consumption" as it is applied by EFSA in the context of the risk assessment for proteins and the requirement or absence of requirement for toxicity testing. The concept of history of safe use should be clarified by adding the reference (Constable et al. 2007). Line 1383: "the isoelectric point". Techniques available to determine the isoelectric point can easily lead to misinterpretation, especially for plant extracts or proteins with higher isoelectric points than 7.5, and there are many other technical challenges (e.g. many proteins require a precise ion strength or divalent ions to reach the native conformation, etc.) In addition, it is unclear if the guidance regarding amino acid sequence comparison

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	requires the full aa-sequence to be analyzed for both proteins. This is a major undertaking and for some proteins (especially for the plant expressed protein) very challenging if possible at all. It should be clarified that not all items listed under the 'For example' sentence are required to show structural, biochemical and functional equivalence and that some of the listed items can only be completed on the microorganism produced proteins. This would be case-by-case for each individual protein. As stated in Lines 1379 through lines 1385, the protein produced in microorganisms is fully characterized to determine its equivalency to the in planta enzyme/protein in cases where sufficient quantities can not be purified from the plant material. It should be made clear that the functional activity characterization is completed on the microorganism produced protein to show equivalence to in planta generated data (e.g. – observed herbicide resistance, production of expected end products, etc.). Based on the text in these lines it does not appear to be the intent of the guidance document to suggest that functional activity characterization be completed on plant purified protein when the characterization is clearly intended for the microorganism produced protein due to difficulties in purifying plant produced protein. The same general comments apply to Lines 1389 through 1394.
7.2.1. Standardized Guidelines for Toxicity Tests	Lines 1364 – 1366: The guidance states that acute toxicity testing is discouraged. Absence of a requirement for acute oral testing signals a lack of harmonization of regulatory requirements around the world. Acute oral testing is still the single best way to identify the NOAEL for a compound and is required by many countries around the world – especially for calculating exposure safety margins in risk assessments. However, the NOAEL (lines 1312-1321, 2835) or UL (2836) is requested in order to derive an appropriate ADI. Does this mean that a subchronic study with proteins should be systematically run and that the calculation will be based on the 28-day NOAEL? The 28-day study is a Tier-3 study (Sjoblad, R.D.et al.). Further the statement contradicts a statement made in the EFSA report on animal feeding studies, published earlier this year where it describes in Section 3.3.1 that the single dose acute oral toxicity 'may be of some value for proteins'. This statement implies that acute toxicity tests provide little value in assessing risks from repeated exposure to a substance. This presupposes that any hazards identified in an acute test would not be useful to predicting risks from repeated exposures. This is not a correct assumption for substances that act through acute mechanisms to produce toxic effects. In regard to proteins, many thousands of plant and animal proteins are consumed in food and pose no risks to humans. It is well known that there are a relatively small subset of proteins that are toxic to various organisms, and the majority of these manifest toxicity through acute mechanisms (Hammond and Cockburn, 2008, Pariza and Johnson, 2001). Bacterial pathogens produce a number of proteins that are acutely toxic to mammals and their modes of action are well known (Alouf and Freer, 1999). Certain plant proteins may also be acutely toxic such as lectins (ricin). If a protein has structural or functional similarity to a known protein toxin, then an appropriate hazard assessment would include an acute high dose test in a mammal. In the United States, the EPA requires an acute high dose test be conducted with plant incorporated insecticides like the Cry proteins since they act acutely to kill insects. Other countries outside of Europe also request acute toxicity tests with proteins introduced into plants to provide added

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	assurances of safety. If there was evidence of toxicity in an acute test, then more toxicity studies would be needed to assess effects from repeated exposures. However, if the protein was not toxic in this test, and its mode of action does not raise safety concerns for non-target organisms, then additional toxicity tests may not be needed. There are exceptions to the acute mode of action for protein toxins such as certain plant lectins, protease inhibitors and the prion family of proteins. These proteins cause adverse effects after repeated exposures. If a protein was related to protease inhibitors or lectins, then repeat dose testing would be more appropriate to assess potential hazards from dietary exposure (Delaney et al., 2008; Hammond and Cockburn, 2008).
7.2.1. Standardized Guidelines for Toxicity Tests	Lines 1349 – 1351: We suggest indicating for studies that are performed according to standardized OECD guidelines, if and when it is acceptable to deviate from the guidelines (eg. shorter duration of the study in some cases, fewer tissues or only relevant tissues be analyzed via pathology, different number of animals). Lines 1357 – 1361: It would be useful if EFSA could provide a few examples of when certain of these protocols might be applied for toxicological testing of GM plant derived food/feed. This is done in section 7.2.5 line 1490, and it would be helpful to refer the reader to this section line 1361.
7.2. Toxicology	General Comments This section has changed considerably from the previous guidance. One of the main changes is the list of studies in Table 1. Significant numbers of animals would be used if these studies were undertaken liberally and without true need and justification. We encourage EFSA to reinforce the commitment to the reduction of the use of animals for testing purposes - the conduct of in vivo toxicity studies should be undertaken with adequate consideration of justification of animal use and welfare commitments. EFSA now discourages the acute oral toxicity study while other international regulatory authorities require it. EFSA reconfirms that a 28-day toxicity study is normally required, while other major international regulatory authorities do not require such a study. Harmonization regarding the utility, applicability and requirements for these studies would be extremely beneficial, especially in the international effort to reduce the numbers of animals used in laboratory testing. An important consideration in this evaluation and revision should be the concept that it is not only possible (and desirable) in some cases to add new criteria or tests, but it should also be desirable to correct or remove part of the earlier guidance document to reflect better understanding of risk analysis. In the step-by-step, or tiered approach, especially in assessment of toxicity and allergenicity, it should be clearly indicated which criteria are purely indicative (sentinel studies) or cut-off criteria. The newly introduced recommendations in the updated guidance document are based on the ‘precautionary principle’ approach, not a scientific evidence-based approach.
7.1.6. Effect of processing	Section D.7.1.6 Effect of processing Lines 1286-1291: We do not see the reason why gene transfer has to be addressed in this context, since it is already assessed for the inserted DNA as required in section D.III.6 of the EFSA guidance document of the scientific panel on GM organisms for the risk assessment of genetically modified plants and derived food and feed. Processing typically produces tens of different foods and feeds, but also numerous

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	by products. It is not feasible to determine DNA and/or protein presence in all the products derived from processing. In addition, processing is often characterized by high temperature and pressure which determine DNA and protein degradation in several instances.
7.1.5. Comparative analysis of agronomic and phenotypic characteristics	Line 1253: typo, should read “Section III, D.7.1.2.” instead of “Section III, D.7.2.”.
7.1.4. Comparative analysis of composition	Lines 1222 - 1223: Please specify the cases when the analysis of cell wall components is warranted. Line 1229: “identified allergens should be studied” in the comparative analysis of composition. Those allergens should be “identified by the OECD consensus documents”. However, the list of allergens is not indicated into the OECD consensus documents. The list of allergens could be found in Allergenonline database (www.allergenonline.org) as this is the only database, which is regularly curated by food allergy experts, and that contain all the food allergenic sequences. Lines 1232 – 1234: Please clarify why the “knowledge of a trait” may trigger the analysis of “downstream metabolites”. Specify “downstream metabolites”. Line 1240: should read “intended” not “intented”. Line 1241: Is 7.1.2. the correct cross reference?
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1131-1139: For each parameter, individual equivalence limits will be calculated, therefore the simultaneous presentation in a single graph is difficult and the labeling of the horizontal axis with values that specify the change (absolute values or even percentage figures) on the natural scale is not possible. Footnotes below the figure between 1139 and 1140: There are too many “i” in the text. Better substitute “…, with its confidence intervals, against: (1) the vertical line showing zero difference…. (2) the vertical lines showing equivalence limits…”. Line 1158-1159: the decision for the 90% confidence interval and the application of the 90% confidence interval in both statistical tests (equivalence test and t-test for mean differences) leads to an increase of the significance level from a = 0.05 to a = 0.1. Consequently, more statistical significant differences will be found by the t-test. However, in hazard approaches small significance level should be defined, to come to reliable and save results in the test. The significance level used should be a = 0.05 or even a = 0.01. Lines 1180-1182: The proposed follow-up action may not be the most appropriate because analyses at the individual site level may fail to reach significance simply because they are much less powerful than the analysis across sites. Testing the significance of the interaction between test material and sites would address the consistency issue directly. (No reference.) Commercial varieties might be quite different to one another, and to the GMO and comparator, in terms of height, time to maturity, attractiveness to

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	pests, etc. Randomising together varieties with very different characteristics could be a problem if this leads to edge effects, so plot size needs to be big enough to overcome this problem. Line 1189-1192: It is not clear how the merging of results like no significant difference and no proof of equivalence or significant difference and proof of equivalence for one compound should be done.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1124-1125: It is not clear why the equivalence limits should be based on the difference between the mean of all commercial varieties and the comparator. Centering the limits on the line of no difference seems more appropriate (NB: the Statistical Considerations document states in lines 833-834 that the precise method of deriving equivalence limits is yet to be finalised). Lines 1126-1127: There may be a flaw in the argument here. Consider a trivial hypothetical example in which two commercial varieties were each tested at the same two locations, giving the following results (each value is the mean of several replicate plots) Loc A Loc B Mean Variety 1 20 30 25 Variety 2 30 18 24 Assuming that plot-to-plot variation at each location was small then it is clear that we have evidence of substantial differences between the two varieties at each location, although the direction of effect is location-dependent. However, the fact that the means across locations are very similar to one another leads to the variance estimate for varieties being very small, which is clearly inappropriate. Thus, it would seem that the proposed method is only appropriate if the true relative performance amongst the commercial varieties does not change from one environment to another, yet there is no reason to assume that this will necessarily be the case. This issue may therefore require more thought. Pooling the variance estimates for variety and the variety x location interaction is one possible solution. Alternatively, if the analysis designed to provide an estimate of variance amongst commercial varieties is separated from that comparing the effects of GMO and comparator then a meaningful estimate of variance for varieties could be obtained by fitting a model with the terms location and variety-within-location (plus block-within-location if appropriate).
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1119 – 1149 (including Figure on p. 33): (1) The description here implies that estimates of the difference between GM crop and comparator, and between comparator and commercial lines, are based on averaging over sites. This raises several questions. If the commercial varieties are not all planted at each site, there is no guarantee that the difference between the commercial lines and comparator will be estimable. Also, it is not clear that the variance component for commercial lines will be estimable if there is substantial unbalance with respect to sites and commercial varieties. For a given trait, if there is a Site*factor(i) interaction, should the tests of difference and equivalence be carried out separately at each site? If so, should the variance component for commercial varieties be estimated separately by site or should a pooled estimate (pooling across sites) be used? Should results (at the minimum, means and ranges) be reported separately by site for each trait regardless of whether there is a Site*factor(i) interaction? (2) The line of zero difference in the Figure is the line representing zero difference

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	between means for GM and comparator. However, the description of how to calculate the upper and lower limits for the test of equivalence does not provide an interval which is symmetric about this line of zero difference (except in the case where the difference between means for commercial lines and comparator is zero). Should the interval for determining equivalence depend on the difference between commercial lines and comparator? Please clarify. It is recommended that results are to be summarized for all components in a figure like that on p.33, except that rows will represent different endpoints rather than different types of outcomes. How is this handled if units of measurement differ across endpoints, or if some endpoints are log transformed, others square root transformed? (3) The figure on p.33 shows the lower and upper equivalence limits as being symmetrical about the line of zero difference but in general this will not be the case if equivalence limits are based on the difference between the mean of all commercial varieties and the comparator. This figure also gives the impression that equivalence limits can be depicted as vertical lines, an impression reinforced in particular by lines 732-733 in the Statistical Considerations document. However, if equivalence limits are to be derived as proposed then their whereabouts will change from one endpoint to another.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1114-1115: In the interests of consistency, it would be helpful to have a clear steer on whether the interaction between test material and sites should be tested and, if so, what should be done in cases where the interaction is significant. Lines 1115 and 1185: It is not clear what is meant by “residual variation”. Please clarify.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Line 1097: The data from commercial varieties are used to define equivalence limits for each endpoint. This is the perfect approach to avoid the generic 20% equivalence limit. The equivalence test is then based on much more realistic scenario than the t-test for mean differences that still works with a fixed p-value for all compounds. One out of ten tests for differences yields a significant result by chance alone in (see also line 1158-1162). Having about 60 to 90 parameters tested and for herbicide traits two comparison between non-GM and GM group this are already 12-18 significant result by chance alone. Line 1100-1111: Precisely how this analysis should be structured is unclear and so provision of a worked example (eg based on SAS code) would be extremely helpful. Investigations suggest that it might prove difficult to come up with an approach that is both technically sound and will provide all of the required information. In particular, estimation of the variety variance is problematic in cases where different commercial varieties are grown at different sites and a term for the location x genotype interaction is included in the model. Whilst the benefits of having commercial varieties grown in the same location as the GMO and comparator are understood, it is not obvious that all conclusions need to be derived from one overall analysis, and consideration should be given to the idea of conducting two separate statistical analyses, one designed to compare

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	the effects of GMO and comparator, the other designed to provide an estimate of variance amongst commercial varieties. This would simplify the comparison of GMO and comparator, and may offer a solution to the problem of deriving a meaningful variance estimate for varieties (See comments below for lines 1126-1127). Moreover, if we remove the need to base equivalence limits on the difference between the mean of all commercial varieties and the comparator (see comment on lines 1124-1125 below), then there would be no need to randomise test entries and commercial varieties together, which would reduce the amount of land to be covered by the field trial release permit. In addition, the statistical model described is not appropriate. It should be limited to the factors test material (treatment) treated as fixed effect and site treated as random effect. In the interests of consistency, it would be helpful to have a clear steer on whether EFSA would prefer sites to be regarded as fixed or random effects, and what they think should be used as an error term for testing the significance of the genotype effect (these two issues are directly related).
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1077 – 1081: A new paragraph is needed to separate the discussion on stacks from the discussion on herbicide-tolerant GM plants. The scientific rationale behind the request for GM test materials, both treated/untreated with the intended herbicide(s), should be provided. If such a scientific rationale exists, such a comparison should only be carried out once either with the single event(s) or with a stack containing the single event(s). For example, in the case where the single events have been treated and untreated with the intended herbicide(s), it would only be necessary to include the stack treated with the intended herbicide(s). Line 1082: New section. Appears to be applicable to ALL comparative analyses. The draft report on general statistical guidance suggests that this is also applicable to animal feeding studies. Clarification needed. Lines 1086-1088: To say that a data transformation may be required and that a log transformation may be appropriate is entirely reasonable. However, throughout the Statistical Considerations document the implication seems to be that all datasets will be log transformed unless there is reason to do otherwise. Extensive experience indicates that compositional study datasets do not normally require a transformation (but neither would use of a log transformation be detrimental). Lines 1092-1093: This change is well accepted. The statistical analysis is performed on the full dataset from all sites; it does not start with the by-site analysis anymore. Line 1093: A comment on how to treat outliers would be helpful.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1064 - 1067: As commercial varieties can be either traditional or GM varieties, this should be specified in the Guidance document. There are crops where traditional varieties adaptable to certain growing regions are not available, like soybean in the US soybean growing regions. Consequently the requested “six different commercial varieties” in this case can be chosen only from commercial GM varieties. Lines 1072-74: These lines do not specify the number of growing season requirement for stacked events. The Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events, [the EFSA Journal (2007) 512, 1-5] in Section 3.2.1- Compositional assessments reads: ”For the stacked events at

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	least one year of field trial data is required,….". This should be stated, similarly to lines 1255-1257 addressing the growing season requirements for agronomic properties and phenotypic characteristics where this requirement is clearly indicated as "over at least one season". Suggest changing line 1073 as follows: "have confirmed that single events do not interact, one additional year of comparison with test…". Lines 1077-1080: The confidence interval approach is fine for comparing just two entries such as GMO and comparator, but how would EFSA like to see results presented in the case of three or more entries?
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lines 1041-43: The approach on the flexibility in the number of years over which field trials are conducted is welcomed. However in order to apply it properly an explanation on the "restricted geographic range" would be necessary. Line 1048: The sentence "At each site the test materials (GM crop and comparator(s)) must be identical" is misleading. If GM crop and comparator(s) are identical there is no reason to compare them. If the intention is to say that GM crop and comparator(s) must be identical at all of the tested sites the sentence should be reformulated. In addition, it is unclear what this statement means. In the same way that the choice of commercial varieties might differ from one location to another in response to different growing conditions, there might also be a need to use different germplasm for the test materials in different locations for the same reason. Lines 1049 – 1053: If different commercial varieties are used at different sites, the data will be unbalanced and estimation of the variance component for the main effect for commercial varieties can be messy. This estimate is needed to get the limits for the equivalence test. Further, historical data and literature data appear to be not relevant any longer. What is the rationale? Comprehensive data sets have been generated via the ILSI crop composition data bases. Lines 1055 - 1061: These recommendations seem to be driven by a desire to have a minimum of 15 df for residual error at each site. However, given that the document makes clear that the key statistical analysis is the analysis across sites then is it not the number of df for the appropriate error term in the combined analysis that matters? Given that the minimum number of sites is specified as eight, then a design made up of GMO, comparator and (the same) three commercial varieties, each with three replicate plots laid out in a randomised complete block design at each of eight sites would give 64 df for residual error and 28 df for the variety x site interaction (which some people might use as the error term). Even the smaller of these two figures would seem to be more than adequate. Thus, a minimum of 4 replicates per site (or 5 if t=5) seems excessive. Furthermore, the number of replications in the case of variety registration testing for agronomic purposes is usually calculated on a level of degrees of freedom of 10. This guideline takes 15 which increased the number of replications. Field trials are normally based on 3 replications given the fact that 8 locations are required, it means 24 reps of data which are estimated as being statistically reliable in agronomy.
7.1.2. Experimental design and statistical	Lines 1028-1030: Differences attributable to genotypes are assessed by comparing composition and agronomic performance of the GM line and its comparator in context of the information available on relevant OECD consensus documents; therefore, we suggest replacing: commercial varieties must be included in the

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analysis of data from field trials for comparative analysis	experimental design of the field trials with commercial varieties could be included in the experimental design of the field trials. When a substantial database of commercial varieties has been established, it may no longer be necessary to incorporate them. It is mentioned in the EFSA Draft Opinion document “Statistical considerations for the safety evaluation of GMOs” (lines 809-810). Lines 1032 – 1034: “It is important that the choice of sites … represents as fully as possible the range of receiving environments where the plant will be grown.” This statement may be incompatible with the statement in line 1048 requiring that “At each site the test material (GM crops and comparators) must be identical.” As a matter of fact in crops like maize or soybean the coverage of all those environments will require the use of several maturity index germplasms differing from one part to the other. In addition, a clarification on the meaning “as fully as possible” in line 1033 would be appreciated. Lines 1036-1038: “Environmental variation is manifest at two scales: site-to-site and year-to year: many years are required…”. Suggest referring to the registration guidelines of commercial varieties that also deal with variety/environment interactions. The EU common catalogue directive estimates that 2 years testing period is sufficient. Line 1038: As it is stated that “… the primary concern is not environmental variation per se, but whether potential differences between test materials vary across environmental conditions …” the mixed model (see lines 1100-1111) used for estimating the variability and the differences should be restricted to these two factors: - Test material/variety with several levels as fixed factor - site as random factor The EFSA guidance on statistical approaches and the updated guidance document for the risk assessment of genetically modified plants and derived food and feed have to be in line with each other. It is further stated that the site by substance interaction should be tested. If sites are random then sites by substance will also be random and there is no test for interaction. It makes no sense to use sites as fixed to test for interaction and then to rerun the model using sites as random because of the affect on the significance level.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Section D.7.1.2. Experimental design and statistical analysis of data from field trials for comparative assessment We believe the general concept that EFSA is trying to introduce - testing for differences and testing for so-called "equivalence", focusing primarily on confidence intervals - is sound. We also think that the proposal regarding how the equivalence limits are set is reasonable. However, in terms of implementation there are a number of details of concern, which are as follows: In any case, applicants should be given the necessary time to adapt to new data requirements which have a major impact on the planning and design of safety studies. Therefore such new requirements should only be applicable after a transition period of at least 3 years. Any retro-active applicability of the new requirements to the dossiers that have already been submitted or that are currently in preparation - the field trials for submissions up to 2011 are currently ongoing - should be avoided.

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	Line 1012: The new concept of equivalence, as referred to in line 1012, may be confused with the principle of substantial equivalence. It would therefore be prudent to use “statistical equivalence” (or another adjective) in order to differentiate from “substantial equivalence”. Line 1009-1023: In the risk assessment it should be demonstrated that the GM crop is in the range of the non-GM comparator and the commercial varieties. Differences between the GM crop and the non-GM comparator are still estimated and described in their variability by confidence intervals. Testing for differences and for equivalence in the same field trial results in problems in interpretation of the results mainly in two possible cases: - No significant difference and no proof of equivalence - significant difference and proof of equivalence In these cases there is absolutely no gain of information from the field trial.
7.1.1. Choice of the comparator	In this guidance document the choice of the comparator is discussed in more detail and we welcome the fact that is not as prescriptive as it was in the stack guidance document. Line 964: “…comparator should have a history of safe use…” In some cases it might be difficult to prove that the conventional line that was used in the transformation and which is the most appropriate comparator has a history of safe use. For this reason we fully support the preceding statement “…do not simply rely on the comparisons with the non GM-material originally used for the genetic modification…” in line 963. However meeting this requirement may require the development of material specifically designed for this purpose (and without commercial value) through backcrossing in out-dated germplasm for example. Lines 976 – 980: Please clarify what is meant by ‘…all information … as web link’, i.e. the documents that need to be web-linked (EFSA opinions/published risk assessments). If EFSA has already risk-assessed the single events, the information relevant for the risk assessment of the stacked product will already be available to EFSA and it is understood that all information provided for this assessment is available through EFSA’s intranet. Line 986: We suggest replacing the word “might” with “would” as normally no safety implications are anticipated with the reduction of the number of stacks. Lines 992 – 995: in line 992, “appropriate comparator” suggests one comparator (from three choices), however plural (“comparators”) follows in line 995. Clarification or correction needed. Line 999: “… not all components may be required …”, which ones? Line 1000: “additional information may be required.”, what information?
7.1. Comparative analysis	Lines 944- 955: This is a new section. Outlines that comparative analysis encompasses composition and agronomic and phenotypic and suggests that the “choice of comparator” applies to both.
6. General recommendations	Lines 939 – 941: Statement on how removal of superfluous DNA simplifies the Risk Assessment. Chapter III.D (Information on the GM plant) should be factual. The assessment in chapter III.D will obviously depend on the complexity of the insertion

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	pattern. A recommendation could be that the insert should be 'as clean as possible'. In any case, suggest moving this chapter to Section II and, as currently written, replace the word recommendation into note. Referring to ACRE makes this note/recommendation trait-specific (ARMs) and has thus to do with the design of the product, not so much with the assessment thereof.
5. Conclusions of molecular characterisation (Sections C and D1-4)	Line 929 - 933: Remove all reference to stacked events and make a separate paragraph in section D.5. that addresses the conclusions needed for stacked events. Paraphrasing from the EFSA Guidance Document on Stacked Events, suggest the following, "For stacked events, the molecular characterisation should focus on the stability of the events combined by crossing, the expression of the traits, and potential interactions that raise any additional safety concerns from previous assessment of the single events." Line 933: The wording "including stacked events" is not appropriate, it should be taken out.
4. Genetic stability of the insert and phenotypic stability of the GM plant	Lines 912 – 927: this section describes genetic and phenotypic stability and only refers to use of southern blots. However, more clarification is needed on phenotypic stability and the use of protein expression data, trait testing. Lines 920 – 921: The prescription of the multiple generations as "normally five" is excessive as no safety rationale has been included to justify the increase in number of generations. This requirement is not in line with the case by case approach. We suggest removing reference to the number of generations and replacing "Applicants should provide data from a representative number of generations". Lines 926 – 927: These direct the reader to section D.2.a. and could be interpreted that extensive analysis is needed to assess genetic stability, in particular for stacks.
3. Information on the expression of the insert	Lines 899 to 904: It should be recognized that the objective of expression analysis is to determine exposure to the newly expressed protein(s). The trial design should be built with that objective in mind, and thus should not be identical to the trial design foreseen for comparative assessment studies, such as compositional analysis. The reference to section D.7.1.2 is therefore not appropriate and should be deleted. Further guidance would be needed on the appropriate number of trials (number of locations and seasons) to produce a relevant range of concentrations of the newly expressed protein(s). It should be clarified whether the statement under lines 902-904 indicates that protein expression data are required from leaves obtained from field grown plants. Lines 905 – 906: It would be good to have further explanation on the rationale as to why and when (i.e. in which situation) information on RNA levels would be required. Lines 907 – 911: please specify what the additional requirements for stacked products would be, what triggers case-by-case and what type could be the 'concerns'?
3. Information on the expression of the insert	Lines 867 – 870: Incorrect. Phenotypic, not expression - expression data does not demonstrate this. Expression data is relevant for margin of safety assessments, however this is not mentioned. Lines 871 – 873: the relevance of post-translational modification under this particular section should be clarified.

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	Line 893: It should be clarified whether this statement indicates that protein expression data should be provided from several seasons. Lines 895 – 896: It would be useful to have further guidance on the type of bioinformatic analysis to be performed. Clarification is needed on the relation of this Chapter with Chapter D2F. Suggest using the same wording as in line 860: "Any ORFs newly created by insertions with contiguous plant genomic DNA"
2. Information on the sequences actually inserted or deleted	Lines 844 – 846: We understand that restriction fragments analysed by Southern blot should also cover the flanking sequences; the probes used will be limited to the insert. To avoid confusion when referring to this section later on in the text when talking about stacks, text should be added: "For stacked events, for which the original events have been fully characterised, molecular characterization, e.g. Southern analysis or PCR, should focus on confirmation of the presence and structure of the inserts of the original events in the stacked event." Lines 861 – 863: need to compare to recently updated databases. Suggest adding "at the time of submission of the application". Lines 864 – 866: Suggest specifying the scenarios that additional assessment may be needed. "If the newly created ORFs have significant similarities with known toxins or allergens assessed by currently accepted methods, further assessment may be needed to complete the information necessary for a comprehensive risk assessment."
3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion	Line 821-822: - The terminology is confusing. Providing the entire sequence of the donor DNA is not appropriate. As long as data are provided demonstrating that all of the DNA intended for insertion into the genome is characterised, then the insert sequence (section III.D.2(e)) is all that is needed for risk assessment purposes. - Proposal for alternative wording: "(a) Annotated plasmid map and table discussing the different elements of the region intended for insertion, incl. any alteration(s) to the donor sequence(s);" Lines 825 – 826: relationship to anti-nutrients has not raised a concern previously. This seems to be a new requirement which could be very difficult to assess. We suggest removing reference to anti-nutrients. Line 829: Clarification needed. We propose to limit to Genus and species.
C. INFORMATION RELATING TO THE GENETIC MODIFICATION	Overall, the clarifications that remove ambiguities and improve the consistency of this section are appreciated. However, some specific comments are listed below. Line 789-791: "…but may depend on the scope of the application". Suggests that source of donor DNA and/or gene function may be viewed differently depending on the scope of the application (import vs cultivation or food/feed vs industrial use). Taking the example cited in the EFSA Draft Opinion on the risk assessment of GM plants for non-food and non-feed purposes (line 376 - line 378), we suggest that a detailed molecular characterisation may not be required if the scope is limited to import of highly processed products). Further clarification (with an example) is needed in the updated guidance document.
B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE	Lines 776-780: This section concerns recipient or parental plants, so "interaction of the GM plant with organisms.." is out of context. Furthermore, this interaction is intensively discussed in Section III.D.8. Suggest replacing the current chapter III.B.7 with Chapter III.B.8.

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APPROPRIATE) PARENTAL PLANTS	
4.2 Issues to be considered for the Risk Assessment of GM Plants	Line 711-720: The order of the list of issues to be considered for the RA of GM plants should follow the order of the “INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED”. Suggest changing the order of the issues as follows: 711 - the characteristics of the donor and recipient organisms; 712 - the genetic modification and its functional consequences; 713 - the compositional, nutritional characteristics; 714 - agronomic characteristics; 715 - the influence of processing on the properties of the food or feed; 716 - the potential for changes in dietary intake; 717 - the potential toxicity and allergenicity of gene products, plant metabolites and the 718 - whole GM plant; 719 - the potential for long-term nutritional impact; 720 - the potential environmental impact;
4.1.1. Hazard identification	Line 660: Adverse effects are generally cited in the context of humans, animals and the environment. “Animals” are missing from this line.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	III.D.11 - Monitoring Line 2306 – 1321: We suggest taking out “In conjunction with human population screening methods currently used by public health organizations (for assessing such elements as incidences of allergic reactions)”. The applicant will collect its information directly from the farmer through farm questionnaires. If a potential unanticipated adverse effect on human health were to occur, it is very likely to be observed by people handling the GMO and thus to be reported in the questionnaire. In case of such observation, the applicant will conduct further analysis of the reported adverse effect to confirm whether it is related to the GMO. III.D.12 - ERA of GM plants containing transformation events combined by conventional breeding Line 2631-2632: ‘the most appropriate comparators”: plural, requires clarification Line 2637-2642: Impact on NTOs should be assessed in one year field trials. The tiered approach should be followed. Line 2644-2652: Impact on NTOs should be assessed in one year field trials. The tiered approach should be followed.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Line 1909: What is “environmental fitness”? Please clarify. Line 2026: “The selective advantage of any transferred trait should be evaluated in different habitats...”. Please clarify. The endpoint should be operational – increased abundance of the wild relative etc. not a vague term like “fitness”. Line 2056: This paragraph should be removed or updated. Again this is the wrong description of “tiered risk assessment”, it is not the sequence in which data is gathered (see comment on line 623).

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	Line 2637: "the potential impact on target organisms should be assessed in one year field trials initially". This statement needs revision as its scientific validity is highly questionable. A synergism study in controlled laboratory conditions has far more power to detect potential effects. Line 2644: Same as above. NTO studies are unnecessary if the synergism study shows no effect. Line 2676: See previous comments on the EFSA's version of tiered testing. Line 2693: Risk assessment should start with what one wants to protect and definitions of harm– it should not start by cataloguing things like cultivation area and routes of exposure. Line 2702: See comment for line 623. Line 2875: Predicting impacts of GMOs on complex ecosystems may be difficult; but that isn't risk assessment. Risk assessment can be very simple – the GMO is no more harmful than a non-GMO. We don't have to predict precisely what each does; only that one isn't different from the other.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Line 636: Tier 3 studies are not exposure studies – they are studies that estimate risk directly without explicitly estimating hazard and exposure. The section should be written so that each tier estimates risk in a particular way (Tier 1 very conservative estimates of hazard and exposure; tier 2 more realistic estimates of hazard and exposure; tier 3 direct measurement of risk etc.). Risk assessments estimate RISK, and it is this that is done in a tiered manner – tiered testing is not the sequential collection of hazard and exposure data. An exception is that it may be possible to estimate risk from exposure only, but only if one can show exposure is negligible such that whatever the hazard, risk is minimal (see comments on Line 658). Line 658: It is too restrictive to say that risk assessments must always begin with hazard identification. It may be possible to demonstrate minimal risk through no exposure. Sections 3 and 4 are very inflexible. They prescribe data that must be collected, without recognising that the data are not the end, but the means to test hypotheses of no harm. There should be flexibility to demonstrate no harm through testing hypotheses with existing data. "Case-by-case" seems to have become "collect the same data for every event", not a flexible system for testing the most suitable risk hypotheses with the most suitable data. There should be more emphasis on what should be protected – without this is not possible to judge whether the data requirements are the most effective way to estimate risk. Line 1907: A general comment is that the document keeps asking for impacts to be described; this is not the purpose of risk assessment, which is intended to determine the probability and magnitude of harm. It is very difficult, and unnecessary, to catalogue all possible changes that may occur following cultivation of the GMO.
3. ENVIRONMENTAL RISK ASSESSMENT AND	Line 623: The tiered approach here does not seem to offer the possibility of stopping testing at lower tiers should results indicate minimal risk. Tiered testing is not the order in which one collects hazard and exposure data; it is the testing of hypotheses of no harm. Testing starts with lower studies that are most likely to falsify hypotheses that are generally applicable, and, if these hypotheses are

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MONITORING	falsified, moves to higher tier studies that test more specific hypotheses (e.g., a laboratory study with high concentrations of protein may be applicable to all crops expressing that protein wherever they are grown, whereas field studies may only be applicable to similar environmental conditions). Line 625: Tier 1 is more than hazard identification – it is the comparison of a measure of hazard with a measure of exposure. Tier 1 Hazard Identification does not necessarily mean exposing organisms to the GM plant AND its products. You can identify hazard by exposing organisms to the GM plant OR its products - whichever is most appropriate to provide useful data for the risk assessment (we do not want to have to both protein AND plant material testing but wish to preserve flexibility). Line 630: Tier 2 studies should not necessarily be studies on trophic layers. Tier 2 studies are similar in concept to tier 1, but the risk is estimated under more realistic conditions.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	General comments: While we understand that the environmental risk assessment guidelines are still under review due to the current self-tasking mandate of EFSA, the text in the present Updated guidance document should be updated so it does not lead to confusion. For example, Section 3 suggests that the risk assessment should comprise studies done at all tiers, whilst Section D.9.5 states that “If first tier tests do not identify sensitivity in exposed species then second and third tier tests may not be required” and Section 12 proposes a minimum of one year field testing, eventually followed by further laboratory tests, where appropriate. While the text in Section D.9.5 is consistent with internationally recognized approaches to tiered risk assessment, the texts in Sections 3 and 12 highlight that there is confusion on what a tiered risk assessment is, compared with a step-by-step data gathering exercise. The step-by-step risk assessment refers to gathering data to assess risk (on hazard and exposure), while the tiered approach refers to an approach that measures risk at each step and allows risk assessors to establish whether or not more data are needed to make an assessment. If more data are considered necessary, then these are generated, either on hazard or on exposure or both, using more refined methods than in previous tiers. The confusion should be removed to allow the applicant to develop a logical risk assessment package, based on a tiered approach. Field trials are only useful if they can be designed to test a clear hypothesis. Field testing should not be a requirement unless lower tier studies indicate the need for this. If additional studies at higher tiers are requested, their purpose should be made clear so they can be designed to respond a specific question to facilitate a regulatory decision, not as a blanket request for detecting potential “unknowns”. More effort on providing clarity on risk assessment endpoints is needed. Overall Sections D.8 and D.9 do not appear to have changed considerably from the approach suggested in the previous guidance. This is disappointing since there have been many proposals and advances in the definition of tiered risk assessment approaches and many publications that could have been incorporated to produce a more up-to-date guidance. In section D.12 there are some points referring to the environmental risk

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	assessment for stacks that XXX and XXX have already made to EFSA. For example, the request for one year of field trials “initially” with target and non-target organisms to assess potential interactions between proteins. These data requirements are clearly in conflict with the tiered risk assessment approach and should be reviewed.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	XXX wishes to express its disappointment that its previous comments on Environmental Risk Assessment were not taken into account.
2.3 Intended and unintended effects	Line 607: Should read “to impact the function” (delete “on”).
2.2 Concept of substantial equivalence or comparative safety assessment	Lines 570-572: In several instances throughout the document, when referring to comparative analysis, the guidance document includes comparative analysis of the molecular characteristics between GM and non-GM. It should however be clarified that molecular characterization is the starting point to structure and conduct the risk assessment and its objective is to characterize the insert and to identify potential unintended effects (Section II.2.3). It should not be considered as a component of the comparative analysis.
2. COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS	Lines 546 – 547 and 570-572: In several instances throughout the document, when referring to comparative analysis, the guidance document includes comparative analysis of the molecular characteristics between GM and non-GM. It should however be clarified that molecular characterization is the starting point to structure and conduct the risk assessment and its objective is to characterize the insert and to identify potential unintended effects (Section II.2.3). It should not be considered as a component of the comparative analysis.
1. INTRODUCTION	Lines 526 – 534: suggest deleting paragraph, since it does not add more clarity to the precise description of the risk assessment process described in the previous paragraphs and in the following section starting at line 654.
I. INTRODUCTION	Line 463 and 470: Suggest changing into: “for the safety assessment of GM plants and derived food and feed”. Line 476 – 478: Since these lines are a title of a subchapter, they should be formatted in bold font style, like other “Interplayers” (lines 420 and 428).
TERMS OF REFERENCE	Line 234: Should read “which was 18 April 2004”
TERMS OF REFERENCE	Line 181: Should read “or derived product” Line 184: Should read “which has applied since April 18, 2004”
TERMS OF REFERENCE	• In terms of the overall risk assessment strategy, XXX would suggest that EFSA revises the methodology proposed for the overall risk assessment. There appears to be some confusion about step by step assessment compared with tiered risk assessment. While the first refers to gathering of data to assess risk (first hazard then exposure...), the later refers to an approach that measures the risk at each step and allows risk assessors to establish whether or not more data is needed to make an assessment. If more data is considered necessary then more data either on hazard or on exposure or both is generated using more refined methods than in previous tiers. • XXX also wishes to bring under the attention that the following Annex

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	documents need to be updated: o Especially Annex III (Format of Technical dossiers) needs to be updated. Since this Annex was not in line with the core text of the previous guidance document, we were requested in the context of some applications to ignore the format described in Annex III, but to follow the format of the core text. To avoid any confusion and unnecessary delays in the future, it is proposed that EFSA aligns this Annex with the core text including the current changes. o Similarly, Annex IV might need to be updated, so that the Summary corresponds better with the text in the Technical dossier (e.g. new sections in III.B; organisation of section III.D.2; last section of D.11) • XXX does not support the idea to capture this guidance document in the legislation in order to make the EFSA guidelines legally binding. Such a legal framework would defy the case-by-case principle of risk assessment and would eliminate all flexibility of a 'guidance' approach.
TERMS OF REFERENCE	• Overall, the updated document requires significant improvements with regard to consistency and clarity, especially in those sections where the EFSA publication on animal feeding trials, the EFSA guidance on stacked products and the outcome of the EFSA self tasking activity on statistics were included into the guidance document from May 2006. Also a better explanation of the underlying rationale and the endpoints is required. • Although XXX welcomes the current clarifications/specifications in the updated guidance document and recognizes that this is to harmonize the comparative assessment approach, at least a three year transition period for implementation of these specific recommendations in study protocols and field trial programs is needed and XXX requests such a transition period to be formally included in the guidelines. The preparation of applications to be submitted until 2011 (incl.) is ongoing and the studies, including field trials to generate the supporting data have been conducted in 2007 and 2008 (2-3 years prior to submission of the actual data). These studies and field trials are designed according to the current standards established by EFSA. A retro-active application of the updated requirements in the guidance document will cause delays of at least 2-3 years in the progress of an application for which the information has been/is being developed. It has to be noted that the protocols for studies and field trials performed until now have been endorsed by numerous EFSA opinions. Moreover, in all instances, use of the existing guideline has provided sufficient information to complete the scientific assessment. • The study design proposed in this guidance differs markedly from the current study design followed to conduct studies for regulatory agencies worldwide. Although we welcome the suggestion that only 1 year of data may be necessary, EFSA is asking that at least three commercial varieties be included in the experimental design to set equivalence limits, each trial must be replicated at a minimum of eight sites that can be grown in a single year or in multiple years but that at least six different commercial varieties must be used as comparators. Obviously this study design will have significant impact on the cost of these studies.
TERMS OF REFERENCE	XXX has integrated all the input from its members into this one document and ensured a common position. XXX Comments on Updated Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed General remarks • EFSA should be commended for providing updated guidance which attempts to

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	add clarity to the specific requirements for performing risk assessment of GM plants and derived food and feed. • One general concern with regard to this guidance is that EFSA has apparently not undertaken an effort to harmonize its guidance and requirements globally. Activities which involve broad global discussion with major regulatory bodies outside of EU, and resulting harmonization of principles and study requirements offer significant benefit to worldwide regulatory bodies, registrants and the public. EFSA should be encouraged to engage in global harmonization activities regarding guidance on risk assessment of GM crops. • Some parts have been rewritten in order to bring some clarifications. Those clarifications are welcome expecting that they will reduce the divergences between different risk assessors. However, it is necessary to keep the right balance between the case-by-case approach taking into account the flexibility and the precision of requirements, as well as the concept of familiarity. The reduction of divergences should be addressed by a closer interaction between expert scientists. • EFSA should also be commended on the rational approach taken for the assessment of stacks in which the higher order stacks are applicable to lower order stacks. However, for overall guidance on stacks, it is currently unclear which document is applicable. Therefore, it would be helpful to clarify whether the EFSA guidance on stacks is now no longer applicable and whether will be withdrawn. • This updated EFSA guidance document must be put in perspective to the draft EFSA report on statistical considerations for the safety evaluation of GMOs. The hypothesis-based status of this draft EFSA report on statistical considerations prevents that the summary recommendations in the updated EFSA guidance document are immediately applicable. The draft EFSA report on statistical considerations clearly expresses the need for validating the new approach. Therefore, it is suggested that industry fully cooperates in the validation of this new approach.
7.2.5. Toxicological testing of the whole GM food/feed	7.2.5 Toxicological testing of the whole GM Food/Feed The testing framework outlined in the proposal is sound however more specific indications should be given as regards the need for further toxicological testing other than the 90-day toxicity studies in rodents. Such study can provide alerts indicating the need for further investigation. For instance functional and /or histological effects on nervous, endocrine, reproductive and immunological tissues/organs should be used as alerts to prompt the need for one- or two-generation studies. Such effects will be especially relevant when they are observed in the absence of significant general toxicity.
7.2.4. Information on natural food and feed constituents	7.2.4. Information on natural food and feed constituents For consumer safety purposes the safety assessment of GM plants and derived food and feed should give due considerations to the variation levels of natural constituents that may pose potential hazards. In particular, on a case by case basis, information should be provided on the following issues: a. a possible variation of the levels of the natural secondary metabolites with known biological activities of concern (e.g. isothiocyanates in Brassica spp., isoflavones in soy) b. a possible variation of the levels of micronutrients that can exert adverse effects on consumers health, associated with an excessive intake (e.g. folates, iodine,

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	selenium) The same general considerations should apply to GM plants used in feeds, as regards both feed tolerance by farm animals and retention of plant components in foods of animal origin.
7.1. Comparative analysis	7.1 Comparative analysis Issues related to the comparative analysis have been improved by the definition of protocol for experimental design and of statistical analysis of data from field trials for comparative analysis. The definition of criteria for selecting an appropriate statistical model in order to evaluate the significance of the observed difference between the GM crop and its comparator has been implemented. Additional guidance on risk assessment of stacked events has been established for the different issues to be considered for the risk characterisation.
OTHER SCIENTIFIC COMMENTS	General comments The updated documents have been significantly improved by the GMO panel establishing an harmonised framework for risk assessment, providing an useful guidance both for the applicants and risk assessors and facilitating the scientific evaluation of the product. In general the requirements to perform an exhaustive environmental monitoring plan (sections 9, 10 and 11) have been detailed and clearly defined. For consumer safety purposes, laboratory and field studies on GM plants should consider, when appropriate, the following issues: a. a possible modification of known plant capability to bioconcentrate certain contaminants (e.g. the known ability of rice to concentrate arsenic) b. a possible increase of natural components with high affinity to contaminants (e.g. lipids such as oils and lipophilic organohalogen compounds) Because this guidance does not cover some particular issues that should be considered on a case-by-case approach in the risk assessment, it should be useful that the application will include also a summary referring to the opinion of the Competent Authority assessing the scientific aspects falling within the scope of specific legislations i.e. the Council Directive 91/414/EEC concerning the placing of plant protection products on the market.
IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL IMPACT	Line 2673-2678: Here a new concept is introduced; ”integrative risk characterization”. Why is it necessary to add the word ”integrative” since the fourth stage of risk assessment has previously been defined as risk characterization (line 688), without being ”integrative”? Is there a difference between ”risk characterization” and ”integrative risk characterization”? If so this should have been defined already in lines 688-720.
7.1.7. Conclusion of the comparativ	Line 1299-1306: We particularly support this point.

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e analysis	
4. Genetic stability of the insert and phenotypic stability of the GM plant	Line 920-922: The necessary number of generations to answer the questions should be studied. In many cases, it should be sufficient if the applicant provides information on generation one and five.
3. Information on the expression of the insert	Line 868-873: Why is it not necessary for the notifier to describe the consequences also of unintended changes?
2. Information on the sequences actually inserted or deleted	Line 860-866: It is also important that the applicant makes an analysis not only of the toxins and allergens that could result from fusion proteins, but also of other potential differences, for instance changes in expression of transcription factors or changes in substrate specificity of enzymes.
1. Description of the trait(s) and characteristics which have been introduced or modified	Line 834-835: We think the applicant should divide the changes into intended changes and unintended changes.
C. INFORMATION RELATING TO THE GENETIC MODIFICATION	Line 792-798: We support the idea that the notifier should state both the DNA intended to be inserted and the DNA that was actually inserted. The words ”inserted in the plant” seem to actually mean ”inserted in the plant genetic material (genome)”, and may have to be changed.
4.2 Issues to be considered for the Risk Assessment of GM Plants	Line 709-720: This list is too much focussed on food and feed. Line 713 should be divided into several different points.
4.2 Issues to be considered for the Risk Assessment of GM Plants	Line 712: should be changed into: ”the genetic modification and its functional consequences, intended as well as unintended”.
4.1 Objectives of the different steps of the safety assessment	Line 654-720: This is a too narrow description and is mainly based on a food and feed perspective. Typical environmental aspects are not considered at all here. For instance, line 671 could be changed into ”… a possible quantification of the toxicological/nutritional potential or the potential for other harmful effects of identified differences…”. We assume that the environmental aspects are supposed to be included in this section since they are actually mentioned in the list (line 713).
4. THE OBJECTIVES OF THE DIFFERENT STEPS	Line 654-657: The heading says ”risk assessment”, but it says ”safety assessment” in the subheading in line 657. So which is it?

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OF THE RISK ASSESSMENT PROCEDURE FOR GM PLANTS AND DERIVED FOOD/FEED AND ISSUES TO BE CONSIDERED	
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Line 623-639: This is about environmental risk studies, not environmental risk assessment. Line 623 therefore needs to be changed.
2.2 Concept of substantial equivalence or comparative safety assessment	Line 564-567: Here it is said that substantial equivalence is the same as comparative safety assessment. However, the concept of safety assessment is used in several places in the document. Is it always equal to substantial equivalence? If not it should be more clearly defined.
2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL	Line 476-478: This sentence sounds as a heading, but is not in bold.
OTHER SCIENTIFIC COMMENTS	D9.5 Interactions of the GM plant with non target organisms In our view, this section needs to expanded to a document by itself as it is one of the most fundamental risks of GM insect resistant crops and often not given enough attention by EFSA because of their (understandable) bias towards food safety. The approach could be similar to that proposed by Andow & Hilbeck. 2004 (Bioscience, 54: 637-649). It needs to include multiple trophic levels and long term considerations. Clear guidance needs to be given to applicants so they do not rely on simple acute direct toxicity tests. This is of such importance, it not clear to us why it is given such little attention. In addition, as new scientific evidence emerges on the potential effects (e.g. for MON810), there shoul dbe some formal arrangement to review the authorisation in light of thsi new evidence.
OTHER SCIENTIFIC COMMENT	Section 9.3 Lns 2017-2034 The potential effects on the population of GM contamination needs

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S	to be addressed (Haygood et al. 2003)
7.2.5. Toxicological testing of the whole GM food/feed	Lns 1530-1533 “If the change observed in a certain parameter falls within this background range of variability, it should still be further considered if there is a dose-response relationship, gender specificity, linkage with other changes, or any plausible cause.” This is definitely an improvement but again, clear criteria are missing. One of xxx''s major disappointments with EFSA is the use of the term “biological relevance” used to negate the need of further investigation of GM foods that show statistical differences. In many cases the use of “biological relevance” is used inappropriately as uncertainty does exist. EFSA and the applicants simply have to admit there are data that raise questions regarding the food safety and there is UNCERTAINTY.
7.2.2. Toxicological testing of newly expressed proteins	Lns 1389-1394 Structural assessment of proteins are necessary here - as these are important (Prescott et al. Journal of Agricultural and Food Chemistry 53: 9023-9030).
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Lns 1014-1015 “In testing for difference the null hypothesis is that there is no difference between the GMO and its comparator against the alternative hypothesis that a difference exists.” We are pleased that EFSA makes this explicit as all too often, comparison to the population is made. D7.1.5 lns 1257-1258“On a case-by-case basis, additional information on agronomic traits of the stacked events may be required from additional field trials.” Again, clear criteria are needed here
3. Information on the expression of the insert	Lns 874-877 requires further assessment of stacked traits (via crossing) only when there is a potential safety issue. However, this cannot be known a priori, so further assessments needed to be performed in all cases. Lns 905-906 “Depending on the nature of the insert, information on the RNA levels could also be required.” Clear criteria are needed here.
2. Information on the sequences actually inserted or deleted	The guidance document says, "A plant to bacteria gene transfer is even less likely if the DNA inserted in the GM plant does not show homology with bacterial DNA". Anyhow, the vector backbone which carries the transgene and the antibiotic resistance marker genes may contain substantially long sequences homologous to bacterial DNA. These vector backbone components (and not only the transgene and/or ARM gene) may provide hot spots for homologous recombination with the bacterial genome leading to the transfer and integration of the transgene and/or the ARM gene (Bensasson et al. 2004). Thus, we recommend that risk assessment concerning homologous DNA sequences should include the whole vector construct and should not only be focused on (even though important) single genetic elements. In addition to that, we would also like to point to the fact that in bacteria non-homologous recombination of DNA fragments is possible and should be taken into account for the risk assessment (Hiom 2003, Thomas and Nielsen 2005). [Bensasson, D., J. L. Boore, and K. M. Nielsen. 2004. Genes without frontiers? Heredity 92:483-9. Hiom, K. 2003. DNA repair: bacteria join in. Curr Biol 13:R28-30. Thomas, C. M., and K. M. Nielsen. 2005. Mechanisms of, and barriers to,

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	horizontal gene transfer between bacteria. Nat Rev Microbiol 3:711-21.]
2. Information on the sequences actually inserted or deleted	We would like to mention that absence or presence of selective pressure has to be carefully evaluated. This is elucidated by following example: The absence of an antibiotic in an environment is no guarantee for the loss or an elimination of the corresponding antibiotic resistance gene from the population (Heinemann et al. 2000). Instead, the antibiotic resistance gene may be maintained because it is co-residing on a genetic element which is coding for a resistance to heavy metals and these heavy metals may be prevailing in the habitat in sufficiently high concentrations (Baker-Austin et al. 2006). Furthermore, Directive 2001/18/EC clearly states in Article 4 (2) that the use of antibiotic resistance markers has to be taken under particular consideration when carrying out an environmental risk assessment. This is an important aspect on the risk assessment process, and thus has to be mentioned in the guidance document. In addition to that, we would also like to refer to the ongoing discussion between the EC and the EMEA on that topic. [Heinemann, J. A., R. G. Ankenbauer, and C. F. Amabile-Cuevas. 2000. Do antibiotics maintain antibiotic resistance? Drug Discovery Today 5:195-204. Baker-Austin, C., M. S. Wright, R. Stepanauskas, and J. V. McArthur. 2006. Co selection of antibiotic and metal resistance. Trends in microbiology 14:176-82.]
2. Information on the sequences actually inserted or deleted	Section III D 9.3 "Potential for gene transfer" puts the focus on the horizontal transfer of the transgene of the GM plant to bacteria. In most of the cases, this transgene is only functional in a eukaryontic (= plant) environment and has no effect on bacterial gene expression if eventually passed over to a bacterial host. Such kinds of transfer events are of minor or no relevance for a risk assessment. However, the fate of the applied antibiotic resistance marker (= ARM) genes, also usually present in the genome of the transgenic plant, is of crucial importance (Goldstein et al. 2005). Originating from a prokaryotic genomic background, and thus usually functional in bacterial receptor strains, ARM genes may lead to reduced therapeutic options in the treatment and prophylaxis of infectious diseases (Bensasson et al. 2004, Morris 2007). Therefore, we would appreciate a clear reference to the phrase "antibiotic resistance marker gene", and the applicants should be required to show that the applied antibiotic resistance marker gene is not transferred from plant material to soil or gut bacteria. [Goldstein, D. A., B. Tinland, L. A. Gilbertson, J. M. Staub, G. A. Bannon, R. E. Goodman, R. L. McCoy, and A. Silvanovich. 2005. Human safety and genetically modified plants: a review of antibiotic resistance markers and future transformation selection technologies. J Appl Microbiol 99:7-23. Bensasson, D., J. L. Boore, and K. M. Nielsen. 2004. Genes without frontiers? Heredity 92:483-9. Morris, K. 2007. Battle against antibiotic resistance is being lost. The Lancet Infectious Diseases 7.]

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V. REFERENCES	The following references were included in comments of the XXX and might be considered in the document: Hilbeck A., Jänsch, S., Meier M., Römbke J. (2008) Analysis and validation of present ecotoxicological test methods and strategies for the risk assessment of genetically modified plants. Federal Agency for Nature Conservation, Bonn - Bad Godesberg: 287 pp. (BfN-Skript 236) http://www.bfn.de/fileadmin/MDB/documents/service/skript236.pdf Andow, D. A. & A. Hilbeck (2004). Science-based risk assessment for nontarget effects of transgenic crops. BioScience 54 (7). 637-649.
OTHER SCIENTIFIC COMMENTS	For the first time, recommendations with regard to stacked events were included to a larger extend in the current draft. Nonetheless, further additions would improve the document. According to the case-specific procedure for risk assessment of GMO, in view of XXX , a full risk analysis must always be submitted also for stacked events. Phrases, which limit this necessity, should be deleted throughout the document. This is especially true, if it would otherwise be up to the applicant to decide upon the necessity to submit case-specific data (e.g., lines 874-877). For instance, data collected for the risk assessment of parental lines can not fully supplement the risk assessment of the stacked event in question, but can provide additional information only. In particular, field surveys on expression of the insert(s), agronomic traits (cf. lines 1254-1258) and on the composition analysis have to be conducted to the same extent (more than one season at several sites) as done with the parental lines (cf. Chapter III D 12). In any case, study designs besides the stacked event should include the respective parental lines and corresponding near-isogenic lines. Toxicological and allergological studies to be included in the application must always be performed with the whole transgenic organism, i.e. with a stacked event. The same is true for studies on the interactions of a stacked event and its biotic environment (e.g., non-target organisms for plants with insect resistance traits). Rationale of this judgement, besides possible synergistic effects of the genes and their expression products are also pleioptropic effects, which occur in stacked events and could be the cause for unexpected effects. In this context, XXX from a scientific point of view considers it unintelligible, that EFSA considers submission of a composition analysis with the highest possible number of single events sufficient for all possible combinations of the related single events (cf. lines 983-991). Protected areas and protected species represent subjects of protection with high value and, therefore in view of XXX , should be considered separately in the risk assessment. Directive 2001/18/EC specifies inter alia „effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations" (Annex II, C.2.1., 3rd point) as effects of the GMO, which have to be assessed. The latter points are relevant, in particular, with regard to rare, endangered and protected species. In total, this forms a basis and a necessity to include both protected species and protected areas in the risk assessment separately. For the assessment of relevant risks, we recommend, like in context with other biotic effects, to collect relevant and robust scientific data. A concept from XXX for the eco-toxicological risk assessment is available. In case of knowledge gaps, XXX argues against cultivation of GMO in and nearby protected areas for reasons of precaution. With regard to guidance on risk management (monitoring) of GMOs the updated EFSA Guidance Document in Chapters D 7.7 and D 11 does not provide any further improvements or changes compared to the earlier version from 2006. In its present state, it does not provide sufficient details on how the monitoring of GMOs should be carried out and, as such, is not helpful for risk managers. In some cases, it lacks substantial issues and falls behind the provisions of Annex VII to Directive

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	2001/18/EC and the guidance provided in Council Decision 2002/811/EC. Further detailed, practical, and concrete guidance is required. We recommend to stronger involve Member State (MS) experts with a professional competence in risk management, environmental protection and environmental monitoring systems to further improve this document. This we consider indispensable, also since both expertise and the responsibility for risk management is primarily with the MS.
IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL IMPACT	The merit of this chapter in order to improve the quality of the risk assessment is questionable. XXX recommends to clarify more thoroughly the aims of this chapter in the introduction (IV. 1) and to revise the chapter entirely. In contrast to the provisions of Directive 2001/18/EC, the current draft update of the EFSA Guidance Document in lines 2685-2692 objects the use of the precautionary principle as a means of risk management, because EFSA considers not to have a mandate for risk management. In contrast to that, however, it is explained in Chapter IV. 4 that risk characterisation should include options for risk management. XXX again criticizes that according to lines 2711-2720, risk characterisation can be finalized even if data, which are considered essential for the risk assessment, are missing or insufficient. Also, in the current draft update it is again indicated (line 2740f), that the list of issues to be considered for risk assessment is 'by no means exhaustive'. In this context, XXX indicates, that aspects to be considered arise, to a large extent, from the information requirements in Chapter III. The points which are listed for the risk characterisation in Chapter VI 3, again show that environmental risk assessment is rated low by EFSA. Instead of providing specific guidance for the different aspects of environmental risk assessment, EFSA confines itself to present effects on the level of ecosystems as complex and – in view of EFSA – unable to be characterized.
7.7. Post-market monitoring of GM food/feed	Firstly, it should be clarified that this chapter refers to post-market monitoring regarding the use of food for human consumption and the feed for animal consumption according to Art 5(3k) / Art 17(3k) of Regulation 1829/2003. It does not refer to monitoring of environmental effects conforming with Annex VII to Directive 2001/18, which is regulated in Art 5(5b) / Art 17(5b) of Regulation 1829/2003 and which is specified in chapter D 11. Therefore we suggest: Line 1865: D 7.7 Post-market monitoring of GM food/feed for human/animal consumption Line 1867: for GM food for human consumption and GM feed for animal consumption according to Art 5(3k) / Art 17(3k) of Regulation 1829/2003. The monitoring of environmental effects of GM food/feed is referred to in chapter D 11. Secondly, we suggest that post-market monitoring of food/feed for human/animal consumption should be obligatory for any application, since human/animal health is an at least as important issue as the monitoring of environmental effects. Therefore, we suggest: Line 1866: Delete "where appropriate". Thirdly, if a GMO is used as food/feed a PMM plan should be included with the dossier, which at minimum should include a General Surveillance plan for the GM product. The details of this PMM plan depend on the extent and type of modification and the outcome of the risk assessment. Further guidance and details are required as to how PMM could be established, for instance with regard to the use of existing food control networks.
7.4. Nutritional assessment of GM food/feed	A subchronic study should always be added to the above mentioned basic data set for nutritional assessment, if the GMP or products made from this GMP are supposed to be used as food or feed, and not only in those cases mentioned in the current draft update of the EFSA Guidance Document. Therefore, a different species of animal should be used in the toxicological study, preferably; the tests should include all parts/products of the GMP, which are supposed to be used as food or feed. It should be amended in this section, that the composition of different

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	test diets used in the same feeding trial should be iso-proteinogenic and iso-energetic. In the list beginning line 1720, natural toxins should be added. Line 1760ff: The sentence “routine livestock feeding trails generally add little to the nutritional assessment of feed from GM plants with agronomic traits” should be deleted, because results of previous studies cannot simple be extrapolated to new GMOs.
V. REFERENCES	Line 3635: Two important parts have been omitted: i) Information on how the GM plant differs from the recipient plant in: reproduction, dissemination, survivability (former point D.4) ii) Any change to the ability of the GM plant to transfer genetic material to other organisms (a) Plant to bacteria gene transfer (b) Plant to plant gene transfer (former point D.6) It is highly recommended to include those in the listing.
7.1. Comparative analysis	XXX refers to its comments provided on the draft "Statistical considerations for the safety evaluation of GMOs" during public consultation by EFSA There comments should also be considered here.
3. Information on the expression of the insert	Lines 868 ff: The document does not acknowledge the importance of the expression analysis for the environmental risk assessment. However, reliable data on the expression of the insert may be a pre-requisite to estimate exposure to non-target organisms. We, therefore, suggest adding the following point in the list: - to quantify the exposure of non-target organisms feeding on the different parts and tissues of the GMP. Lines 881-893: A thorough expression analysis, providing field data covering all representative geographical regions, should be regarded as basic information for any risk assessment of a GMP. Applications including the cultivation of a GMP should present data for all plant tissues. The information on the expression of biocidal or other bioactive compounds should be linked to an exposure assessment combined with the estimation of effects. Therefore, the word ‘effects’ should be replaced by ‘exposure’ in line 883 and the sentence in line 888-890 should be modified in a way that the cultivation alone triggers the submission of expression data from any part of the GMP. Lines 907-911: This paragraph should be complemented with the demand to analyse any changes in expression of the stacked events compared to the single trait events. This will provide needed information on a possible interaction of the stacked genes.
C. INFORMATION RELATING TO THE GENETIC MODIFICATION	The current draft update of the EFSA Guidance Document mentions in line 791 that specific data requirements on molecular characterization depend upon the scope of the application. This aspect is new. Neither is a reason given why it was included, nor is it outlined further, so that the aim and meaning of this text passage remains unclear. It is not comprehensible, why data requirements on molecular characterisation should be subject to the scope of the application. For this reason, XXX suggests to delete the passage in question. The EFSA Guidance Document in its version from 2006, explicitly stipulates, that sequence changes must not only be described, but also evaluated. This was deleted in the update but should be readopted. Regarding the provisions on actually inserted sequences, the current draft update lacks references, that the applicant must show that the actually inserted sequence

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	is the same than the one that was intended to be inserted and that any difference should be evaluated. In the context of the molecular characterization one reference can be found in the current draft update, that risk assessment can possibly be simplified for transgene events with minimal DNA, but without any stipulation what exactly are the criteria and what is meant by a simplified procedure. The earlier version of the EFSA Guidance Document (2006) at the respective position includes a whole passage (EFSA 2006; Chapter II 5, page15), which follows the “best practice” recommendations of ACRE in general and mentions the aspect of antibiotic resistance markers in particular. This passage should be retained entirely.
2. HOW TO CARRY OUT THE RISK CHARACTERISATION	Relating to uncertainties it is important to address them by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies or monitoring GMP in the receiving environment (Cartagena Protocol, 2000). [Cartagena Protocol, Annex III (2000), http://www.cbd.int/biosafety/articles.shtml?a=cpb-43 (URL as of date 17-09-2008)]
1. INTRODUCTION	Line 2691: The view that the application of the precautionary approach is solely in the responsibility of the risk manager is not justified. Although the precautionary approach may be particularly relevant to risk management, it should be considered within a structured approach to the analysis of risks which comprises risk assessment, risk management and risk communication (EC 2000). Precaution and the risk attitude of the risk assessor will also influence how the ERA is shaped and which scientific questions are addressed in the ERA and when deciding if to proceed to the next tier or whether the results of a previous tier allow a particular conclusion on a risk. In addition, it is relevant if risk assessors are also involved in commenting on how or whether risks can be managed or are even involved in decision making (see also Hill et al. 2004). [EC (2000). Communication from the Commission on the precautionary principle. COM (2000) 1. Hill, R., Johnston, S. & C. Sendashonga (2004). Risk assessment and precaution in the biosafety protocol. Reciel 13 (3) 263-269.]
7.4. Nutritional assessment of GM food/feed	In the Draft Guidance Document the compositional equivalence also comprises nutritional equivalence. The nutritional value of a food-/feedstuff however cannot be predicted from analysis of a few plant compounds alone and should be subjected to a separate assessment considering specific nutritional and nutritional physiology aspects.
7.3.1. Assessment of allergenicity of the newly expressed protein	For reasons discussed in detail elsewhere (Spök et al. 2004, 2005, Valenta and Spök 2008), this approach cannot provide sufficiently reliable evidence on allergenic properties of proteins, in particular, on the sensitising/allergenic properties of proteins which have not/hardly ever been consumed by humans/populations. Line 1578: Still missing in this section is the clarification that occupational risks, and respiratory route via dust and aerosols are fully included in the scope of the assessment. Dust and aerosols should be mentioned alongside foods and pollen. Line 1627-1637: Based on this guidance no relevant change of the present practice can be expected, therefore clarification is highly recommended. Present practice only includes amino acid sequence homology comparison, evaluation of the allergenicity of the source and the host plant, and in-vitro digestibility assays.

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	Still, the "second step", a serum screen for identifying allergic reactions in individuals already sensitised to cross reactive proteins is (virtually) not conducted at all. In-vitro digestibility assay, despite considered as additional tests if the IgE binding is negative, is done on a routine basis. The overall guidance to allergenicity assessment is still confusing. The introductory paragraph puts emphasis on a weight of evidence approach. The following sections, however, seem to suggest a step by step procedure, with a second step (targeted serum screen, line 1627) the role of which is unclear: Is the targeted serum screen considered mandatory - regardless of the outcome of the homology comparison and the allergenic characteristics of the source or is this step considered optional? In the latter case it is still unclear what triggers that step. Specific serum screens shall be conducted in case the source organisms have allergenic potential even if the homology search is negative (line 1631). Practice is contradicting this guidance as maize and soybean have been disregarded in the past as allergens despite clinical records of food/respiratory allergy. [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. 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 (URL as of date 17-09-2008) (Also published as vol. 7-04 in the Series of the Federal Ministry of Health and Women. Vienna.) Valenta, R., Spök, A. (2008): Immunogenicity of GM peas. Review of immune effects in mice fed on genetically modified peas and wider impacts for GM risk assessment. BfN series: Bonn: BfN.]
7.3. Allergenicity	For reasons discussed in detail elsewhere (Spök et al. 2004, 2005, Valenta and Spök 2008), this approach cannot provide sufficiently reliable evidence on allergenic properties of proteins, in particular, on the sensitising/allergenic properties of proteins which have not/hardly ever been consumed by humans/populations. Line 1578: Still missing in this section is the clarification that occupational risks, and respiratory route via dust and aerosols are fully included in the scope of the assessment. Dust and aerosols should be mentioned alongside foods and pollen. Line 1627-1637: Based on this guidance no relevant change of the present practice can be expected, therefore clarification is highly recommended. Present practice only includes amino acid sequence homology comparison, evaluation of the allergenicity of the source and the host plant, and in-vitro digestibility assays. Still, the "second step", a serum screen for identifying allergic reactions in individuals already sensitised to cross reactive proteins is (virtually) not conducted at all. In-vitro digestibility assay, despite considered as additional tests if the IgE binding is negative, is done on a routine basis.

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	The overall guidance to allergenicity assessment is still confusing. The introductory paragraph puts emphasis on a weight of evidence approach. The following sections, however, seem to suggest a step by step procedure, with a second step (targeted serum screen, line 1627) the role of which is unclear: Is the targeted serum screen considered mandatory - regardless of the outcome of the homology comparison and the allergenic characteristics of the source or is this step considered optional? In the latter case it is still unclear what triggers that step. Specific serum screens shall be conducted in case the source organisms have allergenic potential even if the homology search is negative (line 1631). Practice is contradicting this guidance as maize and soybean have been disregarded in the past as allergens despite clinical records of food/respiratory allergy. [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. 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 (URL as of date 17-09-2008) (Also published as vol. 7-04 in the Series of the Federal Ministry of Health and Women. Vienna.) Valenta, R., Spök, A. (2008): Immunogenicity of GM peas. Review of immune effects in mice fed on genetically modified peas and wider impacts for GM risk assessment. BfN series: Bonn: BfN.]
III. INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED	Having reviewed almost all applications submitted according to regulation 1829/2003, XXX has very often criticized standards used for data presentation and analysis. Also, missing (cross) references impede the review process of the dossiers to an unnecessary extent. We welcome the guidance given in Annex I of the current draft update of the EFSA Guidance Document which, in theory, should prevent such shortcomings. At the same time, we urge EFSA to put these standards into practice.
4.2 Issues to be considered for the Risk Assessment of GM Plants	The list of points to consider here does not sufficiently represent the needs of the environmental risk assessment. We propose to substitute the item 'the potential environmental impact' by the items listed under Annex II D2 to Directive 2001/18/EG.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Directive 2001/18/EC clearly states that different receiving environments for the GMO must be included in the risk assessment (Art. 4 Abs. 3 and Art. 13 Abs. 2a). In the guidance notes of the commission supplementing Annex II of Directive 2001/18/EC (decision 2002/623/EC) this is substantiated for each documented adverse effect. The implications for other organisms, populations and eco-systems, coming in contact with the GMO in question, must be evaluated. Representativeness of environments should be chosen based upon the area an

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	approval is sought for (hitherto always the entire EU territory) and match with specific questions. In the case that cultivation is applied for, different climatic and geographical conditions, cultivation methods, biogeographical regions and landscape structures should be considered. In this regard, it is necessary to test for a range of conditions to be expected. Data should be delivered at least for key growing areas and in a sufficient amount. The current draft update of the EFSA Guidance Document should provide for specific minimum requirements and criteria for the selection of environments to be considered in the authorization. In the past, missing provisions have resulted in approvals gained mainly on the basis of data from North and South America. This contradicts the provisions mentioned above and the step-by-step principle (preparation of the placing on the market by field trials) included in Directive 2001/18/EC. XXX regards an urgent need for action to urge applicants more rigorously to provide robust scientific data from field trials in the EU as the basis for the risk assessment. This could be done, for instance, in Chapter II of the EFSA document. The “tiered approach” as suggested in lines 623-641 is considered inadequate. The current draft update of the EFSA Guidance Document differentiates between identification of direct effects (tier 1, laboratory), identification of indirect effects (tier 2, laboratory) and exposition-effect assessment (field trials). From the point of view of XXX , the analysis of exposition (exposition pathways and amount of expressed substances) is a necessary pre-requisite to guide subsequent test strategies (e.g., selection of test organisms). The analysis of exposure should always be based on robust expression data of the GMP in question. To test for gene-environment interaction, expression data should be collected in different environments. However, relevant data have not been demanded by EFSA until now. Reference made by EFSA on ‚known exposure levels’ (e.g., lines 629 and 635) suggest that this approach will be retained in the future. XXX is urgently advising EFSA to demand robust and scientific valid exposure data. XXX considers that the principles mentioned in Chapter II 3 should be revised. All references that studies with non-target organisms can be limited to tier 1 should be deleted. In an EFSA workshop in June 2007, different experts have expressed reservations against such a procedure. To integrate different views, XXX suggest a new concept for a stepwise ecotoxicological risk assessment, which stresses the specific characteristics of GMO (Hilbeck et al. 2008). This concept substantiates the provisions of Directive 2001/18/EC and consists of the following components: I: Hazard identification II: Exposition analysis III: Assessment of effects (as interplay between laboratory and field) IV: Risk characterisation Practical experience with enforcement has also shown that special regional characterizations of the Member States (climatic and biogeographical conditions) have not or not sufficiently been included in the approval process until know, even though cultivation had always be applied for (and was approved for) in the entire EU. Thus, it is highly desirable, that this aspect is already taken up in the general principles for the environmental assessment in a way, which requires representative data to be provided.
1. INTRODUCTION	The definitions of the terms “hazard” and “risk” differ from Directive 2001/18/EC and respective guidance notes. In this respect, a “hazard” is defined as the potential of an organism to cause harm to or adverse effects on human health and/or the environment, whereas a “risk” is the combination of the magnitude of the consequences of a hazard and the likelihood of that consequence.
II. PRINCIPLES AND	Risk assessment as outlined in the EFSA Guidance Document is significantly based upon the principles of familiarity and substantial equivalence. In synopsis, the paper meets more the US legislation than the stricter European regulations of

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STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS	Annex II to Directive 2001/18/EC. In particular, the idea of precaution as laid down in Directive 2001/18/EC is not adequately represented. XXX recommends, according to the provisions of Directive 2001/18/EC, to explicitly enshrine the idea of precaution in all areas of the update of the EFSA Guidance Document. Experience with enforcement has shown that, in the view of XXX , too much an emphasis put on familiarity and substantial equivalence (lines 551-579) is a handicap for the case-by-case principle as required by EU legislation. This has often resulted in robust scientific data for the environmental risk assessment (ERA) not being provided. The intention of the EFSA Guidance Document to deduce final proof of safety of GMOs from the concept of substantial equivalence (lines 566-557) must be rejected from a scientific point of view. In its present form, the principles for ERA and monitoring as outlined in Chapter II. 3 of the EFSA Guidance Document do not meet the provisions of Directive 2001/18/EC and require substantial revision. The reference indicating that scientific data on environmental effects can be waived in the case that the application concerns import only (lines 620-622), should be deleted or amended. Losses during transport, storing and processing together with residues and decomposition products of food or feed are environmentally relevant and should be considered in the risk assessment also if the application concerns import only.
1. SCOPE OF THE DOCUMENT	The environmental risk assessment (ERA) is an important aspect when releasing GMPs in the environment. Experiences gained with applications submitted so far show – from an environmental point of view – many severe deficits with regard to the quality and quantity of the provided information. Many Member States (MS) have communicated these deficits to EFSA in recent years. It is, therefore, very unfortunate that necessary amendments with regard to the ERA are delayed for – as is stated in the document – a period of up to two years. This leads to a double standard with regard to food/feed safety as opposed to environmental safety. At the same time, the limited scope of the update is not well communicated in the guidance document which gives the impression that sufficient guidance is given to ALL aspects of the risk assessment (e.g. see line 264). Notwithstanding EFSA's internal decision process on the ERA, it is not clear why stakeholders, including Competent Authorities of MS, are not being asked for comments, at present. In fact, the submission of comments has been prevented by EFSA by disabling the respective entry masks.
I. INTRODUCTION	XXX welcomes the initiative of EFSA to update and specify its guidelines for risk assessment of GMOs. Practical experience when enforcing regulation (EC) No 1829/2003, in the view of XXX shows, that sufficient data upon which a comprehensive environmental risk assessment (ERA) could be based, are usually not submitted with the applications. The same is true for hitherto provided monitoring plans for GMOs. Thus, there is an urgent need, that the data basis submitted with the applications is improved and standardized with regard to ERA and monitoring. XXX , therefore, welcomes the initiative of the Commission to put forward implementing rules concerning the preparation and presentation of dossiers according to article 5, para 7 and article 17, para 7 of regulation (EC) No 1829/2003. As we understand, the current draft update of the EFSA Guidance Document will be the basis of the above-mentioned implementing rules. As with the previous versions of the EFSA document, the evaluation is focused on the safety of human and animal health. At the same time, it becomes obvious that the updated document again does not meet the urgent need for action with regard to standardization of the data basis and the evaluation of the ERA. The current draft update is unlikely to contribute to providing a robust and scientifically valid data base for the ERA as foreseen in Directive 2001/18/EC. Important aspects such as protected areas and protected species were not considered. A decision, whether or not to apply the precautionary principle, can not be left to bodies responsible for

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	risk assessment or even risk management (cf. lines 2690-2692), but is established by European environmental legislation. Principles for ERA as suggested by EFSA and requirements for data to be submitted for authorization lag behind provisions in comparable areas, e.g. for authorization of plant protection products. The guidance notes supplementing Annex II (2002/623/EC) and Annex VII (2002/811/EC) to Directive 2001/18/EC describe key issues on how to conduct ERA and monitoring. Both guidance notes, however, are still kept too general to provide appropriate guidance for applicants. Detailed instructions are required, which data are to be provided for the risk assessment, what needs to be considered for the preparation of a monitoring plan, and which minimum standards have to be complied with from a scientific perspective. In most parts, the EFSA Guidance Document remains too undefined to guide applicants specifically and, thereby, improve the quality of the application documents. Some parts of the document such as Chapter III D 7.1 on ‚comparative analysis' are improved substantially in the draft update; the entire document should be developed further in this direction. In this context, we suggest to include more background information and rationales in the document. With regard to ERA, XXX urges to define a basic data set, which must be provided with the application for any new event. This data set should be used as the basis for an evaluation, upon which a case-by-case decision is made, whether or not additional data and studies are considered necessary. Relevant standards, methods and protocols should be provided for this basic data set. Altogether, in the view of XXX , the current draft update of the EFSA Guidance Document is not suitable to be included in the planned legal text. To ensure, that basic scientific requirements of ERA and environmental monitoring are sufficiently considered, experts in the field of nature and environmental conservation and environmental monitoring should be stronger involved. In this context, we would like to point out, that monitoring is classified on the EU level as an instrument of management and, thus, is primarily in the area of responsibility of the Member States (MS). Hence, EFSA should work together with competent authorities of the MS with regard to relevant guidelines.
7.2.5. Toxicological testing of the whole GM food/feed	Line 1520 onwards: It is appreciated that more attention has been drawn to the implementation of toxicity tests and the interpretation of effects observed thereby. Certainly, it is important that interpretation of effects should be evaluated by independent experts.
7.2.1. Standardized Guidelines for Toxicity Tests	Line 1350: According to Chapter III D.7.2.1, test methods and internationally agreed protocols have to be applied for toxicological testing of GM plant derived food/feed on a case-by-case basis only; no clear instructions are given on when application of certain test protocols is recommended. From our point of view, a repeated dose 28-day study and a 90-day study (subchronic toxicity testing) should be a standard requirement for each GMO and provide a basis for further testing, which might be required depending on the outcome. Furthermore, testing of the whole food and feed beyond a 90-day rodent feeding study, i.e. testing on multiple generations, should not depend on indications which have been made previously for potential of toxicity, but should be carried out, at least if it cannot be excluded that the GM plant derived feed is fed to livestock used for reproduction purposes (breeding sows, dairy cows, etc.).
7.2. Toxicology	Line 1335 onwards: The necessity for testing should not depend on the outcome of the comparative analysis only.
7.2. Toxicology	These uncertainties are not reflected in the presently applied toxicity assessment which heavily relies on indirect evidence and in-vitro studies. The most meaningful studies for toxicity assessment, in-vivo studies, are either not compulsory or aiming at acute toxicity only. Whether in-vivo studies would be required depend on the

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	results of the in-vitro (and the acute tests). A detailed analysis of the toxicity assessment approach revealed weaknesses, in particular, as follows: • The results of homology searches are quickly outdated and interpretation of results is not clear • The test proteins from bacterial sources are not fully representative of the plant-derived protein • Variable practice and questionable relevance of in-vitro digestibility tests • The exposure assessments and risk characterisation do not correspond to normal toxicological practice The analysis also indentified a lack of guidance, in particular, for homology comparisons and in-vitro digestibility tests. These limitations in protein toxicity assessment call for and should guide further improvements of the assessment approach, the methods applied and the testing practice. This is of particular importance with increasing diversity and “novelty” of proteins in the pipeline. Improvement can be achieved by conducting further research, developing and fine-tuning of methods and establishing guidance. As long as the protein toxicity assessment approach has neither been tested nor improved it might be considered to conduct repeated-dose tests according to OECD protocols (with the duration depending on the evidence available) as a standard requirement for assessing novel proteins. The methods available to purify plant proteins and the costs should be more thoroughly reviewed and compared to microbial derived proteins. If expression of the plant protein is sufficiently high (depending on the progress in plant-protein purification methods from plant molecular farming even for lower expression rates) test proteins should be produced from the GM plants. More detailed guidance would be needed on: • presently applied in-vitro digestibility studies (laboratory protocol, chemicals used, validation) • homology searches (databases, parameters, verification, quality assurance, updates) • the robustness of evidence to which one could legitimately refer if relying on the history of consumption/exposure; and on the acceptability of distant relationships between the protein with a history of exposure and the test protein (Clarification would be needed.) • how to differentiate more clearly between toxic and anti-nutritive properties of proteins • the explicit need and methods to assess possible anti-nutritive properties alongside toxic properties of novel proteins Updates in the EFSA Draft only concern the need to provide "up to date" homology comparisons, which was also suggested in the recent study of Spök et al. (2008).

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	[Spök, A.; Dolezel, M; Freigassner, M.; Gaugitsch, H.; Heissenberger, A.; Karner S.; Klade, M.; Proksch, M.; Schneider L.; Treiber, F; Uhl, M. Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs. Forschungsberichte der Sektion IV, Vienna: BMGFJ 1/2008, Part 1 and Part 2. http://www.bmgfj.gv.at/cms/site/attachments/1/5/4/CH0810/CMS1206433032207/forschungsbericht_1-08_-1_teil.pdf (URL as of date 17-09-2008)]
7.2. Toxicology	The section on toxicity assessment has been enriched by introducing more detailed reference to OECD and other guidance documents. The substance of the section on toxicity testing of newly introduced proteins has, however, not been changed. The type of tests and data required will still entirely determined on a case-by-case basis depending on the 'knowledge available with respect to the protein's source, function/activity and history of human/animal consumption. Toxicity testing is still not considered mandatory in case the plant and the new protein has a history of safe consumption by humans and animals. This approach has been criticised before in detail on many occasions. Comments on toxicity assessment of GM plants in general including whole-food toxicity assessment have been provided repeatedly and in detail (e.g. Spök et al. 2004) and are therefore not reiterated here. In the following the comments are therefore focussing on protein toxicity only and are based on Spök et al. (2008): Protein toxicity assessment in the context of GMOs is operating on two main assumptions: i) Orally ingested proteins are not generally associated with safety concerns. ii) If proteins are toxic at all, they are acting by acute mechanisms only. However, the evidential basis supporting these assumptions seems to be rather weak. In the context of regulatory toxicology experience is virtually limited to a small range of proteins comprising of biopesticides and food enzymes. In case of GM crops experience is so far limited to some 40 proteins. Toxic properties of proteins via the oral route and beyond acute toxicity have not been a target of systematic scientific investigations. Evidence which seems to contradict the above mentioned assumptions is derived from the assessment of lectins. In the absence of a significant number of test cases, e.g. proteins with little or no history of human exposure and from other than bacterial sources, it has to be established that the presently applied approach toxicity assessment would be able to detect non-acute adverse effects and to guard against surprises. [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 (URL as of date 17-09-2008) (Also published as vol. 7-04 in the Series of the Federal Ministry of Health and Women. Vienna.) Spök, A.; Dolezel, M; Freigassner, M.; Gaugitsch, H.; Heissenberger, A.; Karner S.; Klade, M.; Proksch, M.; Schneider L.; Treiber, F; Uhl, M. Assessment of Toxic and Ecotoxic Properties of Novel Proteins in GMOs. Forschungsberichte der Sektion

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	IV, Vienna: BMGFJ 1/2008, Part 1 and Part 2. http://www.bmgfj.gv.at/cms/site/attachments/1/5/4/CH0810/CMS1206433032207/forschungsbericht_1-08_-1_teil.pdf (URL as of date 17-09-2008)]
7.1.6. Effect of processing	Lines 1289-1291: In certain cases applicants need to assess the extent to which the processing leads to the concentration of these protein(s) in the final product. Examples for possible cases should be given here.
7.1.4. Comparative analysis of composition	Lines 1228-1234: The Draft is still vague about the selection of compounds: The OECD consensus documents are considered to provide a minimum list of compounds for analysis while at the same time other than key nutrients, key toxicants, and anti-nutrients and allergens identified by the OECD documents "may be included … on a case by case basis". Depending on the interpretation, allergens seem to be included in the minimum list. However, recent praxis has failed to identify the concentration of allergens in certain plants. Does this pertain to major plant allergens only? Clues should be provided in which cases the measurement of the concentration of plant allergens is considered necessary.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Line 1064 onwards: It is unclear whether the recommendation that trials may be conducted in a single year or spread over multiple years refers to trials at a single site or to the entire set of trials. The definition of a "site" chosen for field trials is unclear. The exact geographical position should be indicated (e.g. geographical coordinates) and a justification/prove that the chosen site is representative for a particular growing area of a particular crop should be required. It is also unclear, in what cases field trials for more than one growing season are requested. The criteria that this is only the case if "the choice of sites is over a very restricted geographic range" should be further specified. In general, all field trials at a specific site should be conducted for more than one season only (see above). A requirement should be included that field trials should take place in representative European environments if cultivation is included in the scope of the notification. Further guidance is needed with respect to the selection of such representative environments. Lines 1072-1081: The whole paragraph concerning the comparative approach of GM plants containing stacked events is imprecise and not comprehensible. Therefore, we would like to ask for clarification. Line 1082 onwards: The presentation of the results of field trial data on plant composition should also include a site-specific analysis and differences observed at particular sites should be addressed and discussed with respect to their environmental relevance. Lines 1160-1162: Significant different results obtained by the experimental set-up described in this document should always lead to further investigation and can not be rendered irrelevant by a discussion or comparison to literature data. It remains completely unclear what should be considered "biologically relevant". If the parameter under investigation or a significant difference in the parameter is biologically not relevant, why should it be measured at all? Line 1180 to 1183: The document "recommends" further statistical assessment in case of significant difference and/or lack of equivalence. This should not be a recommendation but a requirement. Statistical analysis, however necessary, can only be the first step in further investigations. In most cases it will be necessary to obtain additional experimental data. A comparison to literature data, which will only widen the variability, and render the experimental set-up described in the document useless, should explicitly be discouraged in the document.

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7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Line 1003 onwards: As far as the experimental design is concerned, it is appreciated that the respective chapter has been revised. Further issues, which may be missing in the draft document, are discussed in the draft report on statistical guidance for the safety evaluation of GMOs. However, both in the draft and the statistical guidance document for field testing a minimum time-period of one year only is considered appropriate (lines 1041, 1064), although recommendation of the use of random effects to model possibly environmental factors (sites, years) is particularly addressed in the statistical guidance document (see Draft report on statistical considerations, line 988). The minimum time-period for field trials should be at least 2 seasons in order to have the opportunity to analyse possible problems with the test design and thus avoid them in the following year. Furthermore, one season may not be representative due to extreme weather or other environmental conditions at a specific site. At last, the commercial varieties should correspond to varieties actually used in practice/available for commercial growing at the respective sites where the field trials are located.
7.1.1. Choice of the comparator	Line 983 onwards: The validity of the assessment of a "higher" stacked event for the risk assessment of a "lower" stacked event, as proposed in the document at hand, cannot be regarded as justified, both scientifically and legally. Each stacked event represents an individual case and must be subject to a separate risk assessment, including the evaluation of potential synergistic or antagonistic interaction effects of its gene products as well as possible unintended effects. In addition, the suggestion contained in the draft contrasts with previous EFSA Guidance, stating that the non-GM equivalent and if not possible, the GM parental lines should be used as comparator for stacked events (EFSA 2006, p. 23). In the guidance document on stacked events (EFSA 2007) the "GM parental materials and non-transgenic genotype with comparable genetic background to the GMO containing the stacked events" are required as comparators. This guidance document should thus be consistent with existing guidance. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100. EFSA Guidance Document for the risk assessment of genetically modified plants containing stacked transformation events by the Scientific Panel on Genetically Modified Organisms (GMO). Adopted on 16 May 2007 (Question No EFSA-Q-2003-005D). http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753824_1178623591786.htm (URL as of date 17-09-2008)]
7.1.1. Choice of the comparator	Lines 957-967: Chapter 7.1.1. does not give clear instructions on: i) which generation(s) should be used for back-crossing, and ii) which plants (GM, non-GM) should be back-crossed to generate most appropriate comparators. For example, if GM plants and non-GM plants are crossed and GM plants of the second generation (F2) are then compared to non-GM plants, according to Mendelian inheritance patterns, epigenetic modification caused by GM plants in the parental generation (F1) may get lost. Furthermore, we would like to point out that, according to the basic rules of a reasonable comparative approach and to this Draft EFSA Guidance Document,

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	comparators should have a history of safe use. It should be specified: i) how this history would have to be defined? ii) what plant varieties can be included for that history? iii) what plant varieties will be excluded? [
7.1. Comparative analysis	Lines 957-967: Chapter 7.1.1. does not give clear instructions on: i) which generation(s) should be used for back-crossing, and ii) which plants (GM, non-GM) should be back-crossed to generate most appropriate comparators. For example, if GM plants and non-GM plants are crossed and GM plants of the second generation (F2) are then compared to non-GM plants, according to Mendelian inheritance patterns, epigenetic modification caused by GM plants in the parental generation (F1) may get lost. Furthermore, we would like to point out that, according to the basic rules of a reasonable comparative approach and to this Draft EFSA Guidance Document, comparators should have a history of safe use. It should be specified: i) how this history would have to be defined? ii) what plant varieties can be included for that history? iii) what plant varieties will be excluded?
7.1.1. Choice of the comparator	The concept of "equivalence limits" (EL) is introduced for the proof of equivalence (not for the proof of difference). EL will be established entirely based on the variability of the commercial varieties (1111) which must be included in the field test "in sufficient numbers to ensure an adequate estimate of the variability required to set the equivalence limits" (1028-1030). In 1049 three commercial varieties per site are defined as minimum. Eight sites are considered minimum with a minimum of six different commercial varieties over the entire set of trials (1062-1067). EL: ‘difference between the mean of all commercial varieties and the comparator plus or minus the product of 1,96 times the estimated standard deviation of the random effects for the commercial varieties in the mixed model’ (1125-1128). For field trials (1009-1043) no reference to literature data is being made. Potential outcomes of the proof of equivalence are clustered into four different outcomes: equivalence, "probable equivalence", "probable non-equivalence", and "non-equivalence" depending how exactly the mean and confidence intervals of each endpoint lie with respect to the EL (1163-1178).
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	The concept of "equivalence limits" (EL) is introduced for the proof of equivalence (not for the proof of difference). EL will be established entirely based on the variability of the commercial varieties (1111) which must be included in the field test "in sufficient numbers to ensure an adequate estimate of the variability required to set the equivalence limits" (1028-1030). In 1049 three commercial varieties per site are defined as minimum. Eight sites are considered minimum with a minimum of six different commercial varieties over the entire set of trials (1062-1067). EL: ‘difference between the mean of all commercial varieties and the comparator

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	plus or minus the product of 1,96 times the estimated standard deviation of the random effects for the commercial varieties in the mixed model' (1125-1128). For field trials (1009-1043) no reference to literature data is being made. Potential outcomes of the proof of equivalence are clustered into four different outcomes: equivalence, "probable equivalence", "probable non-equivalence", and "non-equivalence" depending how exactly the mean and confidence intervals of each endpoint lie with respect to the EL (1163-1178).
7.1.1. Choice of the comparator	To detect possible differences between GM-plants and traditionally cultivated crops which have a history of safe use comparative analysis that provides reliable, significant data is essential. The significance of the comparative analysis depends on the choice of the right comparators, the experimental design, the statistical analysis and its interpretation. Line 956 onwards: A check of the measured/identified differences between the GM crop and the isogenic counterpart against literature or OECD data of variations in individual plant compounds is no longer recommended. Instead the concept of 'equivalence limits' is introduced which seems to be the sole bases for assessing the relevance of identified differences between the GM crop and conventional counterpart. Elsewhere (1108-1110) proximates, key macro- and micro nutrients, anti-nutritional compounds, and natural toxins are considered a minimum set of compounds. The guidance should explicitly address this change and explain the reasons.
6. General recommendations	Lines 940-941: It is unclear why the risk assessment may be simplified for certain cases. It should be specified for which cases this simplification may apply, and with respect to which GM events and to what extent the ERA may be minimised.
OTHER SCIENTIFIC COMMENTS	9 POTENTIAL CHANGES IN THE INTERACTIONS OF THE GM PLANT WITH THE BIOTIC ENVIRONMENT. For consumer safety purposes, laboratory and field studies on GM plants should consider, when appropriate, the following issues: a. a possible modification of known plant capability to bioconcentrate certain contaminants (e.g. the known ability of rice to concentrate arsenic) b. a possible increase of natural components with high affinity to contaminants (e.g. lipids such as oils and lipophilic organohalogen compounds)
4. Genetic stability of the insert and phenotypic stability of the GM plant	Lines 920-922: An adequate number of individual plants should be analysed for genetic stability. The number of individuals analysed should be chosen with regard to an appropriate level of stability. If methods are used that are applicable for large numbers, e.g. PCR, the number of samples should be taken, according to regulations of UPOV and ISTA for testing the homogeneity and purity of seeds. For other methods like sequencing, which are more labour-intensive, a lower number of samples may be taken. Lines 926-927: The molecular methods described in section D.2.a. are too imprecise to sufficiently characterise the genetic stability of transgenic plants. It should be analysed whether alterations or rearrangements in the genome (single point mutations, recombinations, deletions, translocations, etc.) occur in transgenic plants. To achieve this, methods which were used for the characterisation of the internal structure of the insertions shall be used (PCR, Southern Blot). However, minute differences in length can only be seen if the PCR product is sufficiently small. Thus, the construct amplification should be divided into several segments which have to be amplified separately (as described in Singh et al. 2007). Additionally, it is necessary to analyse the mutation rate of the construct via

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	sequencing, especially the 5'' and the 3'' transitions into the genome because small mutations can lead to changes in the ORF and to irregularities in the genome (Rosati et al. 2008). The same requirements shall apply for the characterisation of stacked events. [Rosati, A., Bogani, P., Santarlasci, A., Buiatti, M. (2008). Characterisation of 3'' Transgene Insertion Site and Derived mRNAs in MON810 YieldGard Maize. Plant Molecular Biology 67, 271-281. Singh C.K., Ojha A., Kamle S., Kachru D.N. (2007). Assessment of cry1Ab transgene cassette in commercial Bt corn MON810: Gene, Event, Construct & GMO specific concurrent characterization http://www.natureprotocols.com/2007/10/23/assessment_of_cry1ab_transgene.php (URL as of date 17-09-2008)]
7.2.5. Toxicological testing of the whole GM food/feed	7.2.5 Toxicological testing of the whole GM Food/Feed The testing framework outlined in the proposal is sound however more specific indications should be given as regards the need for further toxicological testing other than the 90-day toxicity studies in rodents. Such study can provide alerts indicating the need for further investigation. For instance functional and /or histological effects on nervous, endocrine, reproductive and immunological tissues/organs should be used as alerts to prompt the need for one- or two-generation studies. Such effects will be especially relevant when they are observed in the absence of significant general toxicity.
7.2.4. Information on natural food and feed constituents	7.2.4. Information on natural food and feed constituents For consumer safety purposes the safety assessment of GM plants and derived food and feed should give due considerations to the variation levels of natural constituents that may pose potential hazards. In particular, on a case by case basis, information should be provided on the following issues: a. a possible variation of the levels of the natural secondary metabolites with known biological activities of concern (e.g. isothiocyanates in Brassica spp., isoflavones in soy) b. a possible variation of the levels of micronutrients that can exert adverse effects on consumers health, associated with an excessive intake (e.g. folates, iodine, selenium) The same general considerations should apply to GM plants used in feeds, as regards both feed tolerance by farm animals and retention of plant components in foods of animal origin.
3. Information on the expression of the insert	Line 868: Regarding the information on the expression of the insert applicants should account for following issues: • If stacking of events has occurred, it should be tested whether the proteins can interact or at least influence each other. • It should be tested whether the expression levels between individual plants can vary (Nguyen and Jehle 2007). • It should be tested whether the expression levels between different areas of cultivation can vary (Nguyen and Jehle 2007).

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	[Nguyen H.T. & J.A. Jehle (2007): Quantitative analysis of the seasonal and tissue-specific expression of Cry1Ab in transgenic maize Mon810. Journal of Plant Diseases and Protection, 114(2): 820-87.] Line 878: This section should contain a phrase clearly expressing that "quantitative information about insert-specific protein as well as mRNA expression must be provided". It is also necessary to check the insert-specific mRNA for alternative splice sites. Lines 881-886: If relevant (e.g. insect resistant GMPs), the selected developmental stages for the assessment of developmental expression should correspond to the biological characteristics of the respective target organism(s), such as timing of occurrence of the target organism on the GMP. This information is relevant for any IRM strategy applied for insect resistant GMPs. Guidance should also include relevant growth stages of a particular crop species to be selected for expression analysis in order to enhance comparability between GM crops of the same species. Lines 895-896: In case potential fusion proteins are identified during the molecular characterisation, the expression of these fusion proteins should be further investigated experimentally. Lines 897-898: The following requirements should be introduced concerning the methods for assessing expression: • Use of standardised sampling procedures • Use of standardised methods for all tests of expression of transgene products conducted in a specific notification. • Validation of detection methods for certain transgene products, like specific Bt toxins, which are expressed in a number of different GMPs (including stacked events constructed from these GMPs) to enhance comparability of expression results between individual experiments reported in a notification and between experiments from different notifications of GMOs, which are expressing the same transgenic products. Lines 899-906: The requirement concerning the establishment of a range for the expression of transgenic components is supported. The assessment should be conducted under conditions which are representative for commercial application of the GMPs and should cover more than one growing season.
7.1. Comparative analysis	7.1 Comparative analysis Issues related to the comparative analysis have been improved by the definition of protocol for experimental design and of statistical analysis of data from field trials for comparative analysis. The definition of criteria for selecting an appropriate statistical model in order to evaluate the significance of the observed difference between the GM crop and its comparator has been implemented. Additional guidance on risk assessment of stacked events has been established for the different issues to be considered for the risk characterisation.
3. Information on the	Line 868: Regarding the information on the expression of the insert applicants should account for following issues:

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expression of the insert	• If stacking of events has occurred, it should be tested whether the proteins can interact or at least influence each other. • It should be tested whether the expression levels between individual plants can vary (Nguyen and Jehle 2007). • It should be tested whether the expression levels between different areas of cultivation can vary (Nguyen and Jehle 2007). Line 878: This section should contain a phrase clearly expressing that "quantitative information about insert-specific protein as well as mRNA expression must be provided". It is also necessary to check the insert-specific mRNA for alternative splice sites. Lines 881-886: If relevant (e.g. insect resistant GMPs), the selected developmental stages for the assessment of developmental expression should correspond to the biological characteristics of the respective target organism(s), such as timing of occurrence of the target organism on the GMP. This information is relevant for any IRM strategy applied for insect resistant GMPs. Guidance should also include relevant growth stages of a particular crop species to be selected for expression analysis in order to enhance comparability between GM crops of the same species. Lines 895-896: In case potential fusion proteins are identified during the molecular characterisation, the expression of these fusion proteins should be further investigated experimentally. Lines 897-898: The following requirements should be introduced concerning the methods for assessing expression: • Use of standardised sampling procedures • Use of standardised methods for all tests of expression of transgene products conducted in a specific notification. • Validation of detection methods for certain transgene products, like specific Bt toxins, which are expressed in a number of different GMPs (including stacked events constructed from these GMPs) to enhance comparability of expression results between individual experiments reported in a notification and between experiments from different notifications of GMOs, which are expressing the same transgenic products. Lines 899-906: The requirement concerning the establishment of a range for the expression of transgenic components is supported. The assessment should be conducted under conditions which are representative for commercial application of the GMPs and should cover more than one growing season.
2. Information on the sequences actually inserted or deleted	Lines 860-866: Any chimeric ORFs identified for transgenic inserts in a certain GMP should be further assessed. This comprises, on the one hand, ORFs for potential fusion proteins situated at the borders of transgenic inserts to native genomic plant sequences and ORFs for potential, newly expressed proteins resulting from rearrangements to the genomic border sequences. On the other hand, ORFs within transgenic inserts created by insertion of more than one copy of the insert and/or additional fragments of transgenic sequences need to be assessed. We recommend that following issues may be added:

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	• Whether DNA from mitochondria or chloroplasts flanks the insert, as it can occur with biolistic delivery methods. The sequence adjacent to the insertion point in the parent plant should be used to demonstrate that the insertion into the genome was successful. • The sequences that are flanking the insert should be tested for homology to regulatory regions. • It should be investigated whether parts of the vector or the insert have migrated to other regions of the genome as well (Rosati et al. 2008). Also, the following general requirements should be introduced for the submission of molecular data: • All transgenic insertions present in a GMP as well as the copy number of (functional and non-functional) inserts should be assessed by different and complementary methods to achieve robust evidence. • High quality data shall be submitted with regard to two issues: the chosen test designs should ensure direct conclusiveness of data and the quality of presentation of data should be good enough to decide whether the results unambiguously support the conclusions drawn by the notifier. • For the comparison of native genomic sequences present at the 5'' and 3'' junctions to the transgenic insertions, an analysis of the respective loci in the genome of the unmodified recipient plant is required. Guidance for molecular characterisation should include the requirement for such an analysis. This requirement is particularly important in case no sequence data for the respective loci have been reported in a certain crop species. [Rosati, A., Bogani, P., Santarlasci, A., Buiatti, M. (2008). Characterisation of 3'' Transgene Insertion Site and Derived mRNAs in MON810 YieldGard Maize. Plant Molecular Biology 67, 271-281.]
7.1.5. Comparative analysis of agronomic and phenotypic characteristics	r. 1248-1253 EFSA states: “The comparison between the GM plants and their most appropriate comparators should address also plant biology and agronomic traits, including common breeding parameters (e.g. yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress). The protocols of these field trials should follow the specifications made under Section III, D 7.23”. However, it is not it is not clear how the criteria mentioned in section III D 7.2 (toxicology) apply to data on phenotypic and morphological comparisons.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	EFSA states in their document on statistical considerations for the safety evaluation of GMO’s, in which the experimental design and statistical analysis of data from field trials for comparative analysis is described: “The Working Group emphasizes that the current report represents a first analysis of approaches and limitations, and only after testing on several datasets it may be possible to make more specific proposals in a second report”. However, in the updated guidance the approach taken in this first analysis is presented as being requirements for experiments. We consider this as a contradiction from EFSA and we would like clarification on this issue.
7.1.2. Experimental design	In the description of the requirements for field trials and the statistical analysis there is only mentioning of a simple case: single events and its comparators (non-GM and commercial varieties). Since it is expected that in future more applications

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and statistical analysis of data from field trials for comparative analysis	will be filed for stacks with a high number of events, it should be made clear which comparators should be used in the trials for these stacks. It is suggested that examples will be included in the guidance that elaborate on these situations.
7.1.1. Choice of the comparator	r. 983-991 EFSA states “In the case of events stacked by conventional crossing the GMO Panel is aware that there is likely to be a move towards further increases in the numbers of events in GM stacks. As long as each event in the highest number of stacked events has been risk assessed, the risk assessment might also be applicable to stacks containing fewer of these events. Thus a single risk assessment for the highest number of stacked events could cover all combinations with fewer of these events. However, applicants need to take into account the potential impact of any reduction in the number of events involved and provide scientific reasons why specific data on the stacked events with a lower combination of events are not included” The NL would like to request EFSA to be more explicit on this matter. What comparators are to be used in case of stacks? What comparators to use for stacks when comparing gene expression, agronomical characteristics, phenotypical traits or when performing a comparative analysis? Can EFSA give more guidance in this respect?
3. Information on the expression of the insert	r. 902-904. It is stated: “Protein expression data should be related to the conditions in which the crop is grown and should be carried out in parallel with compositional analysis as specified in Section 7.1.2”. Can EFSA explain what is meant by ‘ to be carried out in parallel with…..’?
B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS	The adjustments of the guidance focus seem only to focus on food and feed aspects and not on environmental issues. For example in the explanation on information requirements for the recipient plants (r. 750-757) there is only mentioning of expression of toxins or allergens, but not of environmental aspects of the plant like potential for out crossing. Is this part of the guidance updated again in a later stage when the environmental part of the guidance is also updated?
2. Information on the sequences actually inserted or deleted	Lines 850-853: For inserts which are localized in the nuclear genome, the chromosomal location should be assessed. Reference should be made to direct methods for demonstrating nuclear localization, which can give indications on the chromosomal location of inserts.
I. INTRODUCTION	General The document on stacked event is integrated in the text, but not in a consistent way and throughout all sections of the document. For example: stacked events are not mentioned in the molecular characterization part of the guidance, neither in the part on toxicology. Furthermore it is not always made clear throughout the text when there is mentioning of stacked events if it concerns stacked events for which the single events are already assessed on their safety or not. This makes quite some difference in the information requirements for safety. Our suggestion is to take into account in the guidance itself only stacks of which the single events are not (yet) approved (or stacks not generated by traditional breeding) and to include the information requirements for the other stacks in an annex.
2. Information on the	Lines 840-846: Relating to the information on the sequences inserted or deleted it would be useful if quantitative information about the copy number of inserts and

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sequences actually inserted or deleted	other important genetic elements will be obtained by using quantitative real time PCR. qPCR is currently state of the art for retrieving reliable quantitative genetic data (Providenti et al. 2006, Shimizu et al. 2008, Skulj et al. 2008, Wilhelm and Pingoud 2003). Southern analyses should also be supported by sequence data, wherever possible. Additionally, the analysis by Southern Blot for all detectable inserts (complete as well as partial) should include an analysis of the sensitivity of the method. Such an analysis should identify the minimal amount of sequences which can be detected by the method used. The requirement to submit information on the sensitivity of the method should be added to the guidance. [Providenti, M. A., J. M. O''Brien, R. J. Ewing, E. S. Paterson, and M. L. Smith. 2006. The copy-number of plasmids and other genetic elements can be determined by SYBR-Green-based quantitative real-time PCR. Journal of microbiological methods 65:476-87. Shimizu, E., H. Kato, Y. Nakagawa, T. Kodama, S. Futo, Y. Minegishi, T. Watanabe, H. Akiyama, R. Teshima, S. Furui, A. Hino, and K. Kitta. 2008. Development of a screening method for genetically modified soybean by plasmid-based quantitative competitive polymerase chain reaction. Journal of agricultural and food chemistry 56:5521-7. Skulj, M., V. Okrslar, S. Jalen, S. Jevsevar, P. Slanc, B. Strukelj, and V. Menart. 2008. Improved determination of plasmid copy number using quantitative real-time PCR for monitoring fermentation processes. Microbial cell factories 7:6. Wilhelm, J., and A. Pingoud. 2003. Real-time polymerase chain reaction. Chembiochem 4:1120-8.]
I. INTRODUCTION	General The guidance document is still in the process of updating, as is also mentioned by EFSA in the guidance itself. Reports of several self tasking activities, some quite important for the guidance (for example on environmental aspects), still have to be included. Would it not make more sense to wait for an overall update of the guidance including all aspects of the risk assessment (including the environmental aspects)?
2. Information on the sequences actually inserted or deleted	Line 837: According to Directive 2001/18/EC (Annex II C.2), "Any characteristics of the GMOs linked to the genetic modification that may result in adverse effects on human health or the environment shall be identified". Therefore, information on the sequences actually inserted or deleted has to be provided to assess the intended and the unintended effects (any characteristics) that result of the genetic modification, not only to assess whether unintended effects may occur.
1. Description of the trait(s) and characteristics which have been introduced or modified	Line 832-835: According to the draft document, only information on the trait and the changes that it makes to the plant phenotype shall be submitted. The EFSA Guidance Document (EFSA, 2006) specifies that further information needs to be submitted under this item (quantification of the phenotypic modifications, targets of the trait, sensitivity of non-targets, purpose of the modifications, use of the GMO, changes in plant composition, management, cultivation, deployment, geographic range and end use). The draft document should assure that this specific information is included in other respective chapters of the notification. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]

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3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion	Line 821: As specified in the EFSA Guidance Document (EFSA 2006) an assessment of the alterations to the donor DNA sequences present in the sequences which are transferred during the construction of the GMP should be required. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]
2. Nature and source of vector used	Line 807: It would be preferable if the applicant will not only provide a physical map of the vector but also the complete sequence of this (modified) vector. This could be economically achieved by combining sequence information provided by the manufacturer of the vector and in-house sequencing of the inserts and bordering regions. Complete sequencing of the final vector construct would be optimal but probably is not adequate due to resource limitations. We would also recommend that the applicant provides data about possible epigenetic effects introduced by the vector/insert construct after the successful transfer into the plant genome (e.g. post transcriptional genomic silencing, etc.) (Horiguchi, 2004). [Horiguchi, G. 2004. RNA silencing in plants: a shortcut to functional analysis. Differentiation 72:65-73.]
1. Description of the methods used for the genetic modification	Line 804: It should be mentioned that the use of carrier DNA should trigger additional scrutiny for extraneous sequences present in the GMP and that the use of carrier DNA is discouraged (SBB 2003). Furthermore, advice should be given that the applicant should also provide information about helper plasmids if used during the genetic transformation process. [SBB (Section of Biosafety and Biotechnology) (2003): Guidelines for molecular characterization of genetically modified higher plants to be placed on the market. http://www.biosafety.be/gmcropff/EN/TP/partC/GuidePartC.pdf (URL as of date 17-09-2008)]
1. Description of the methods used for the genetic modification	Lines 801-802: The addition of item b) (information regarding the recipient plant material) to the list of information items is supported, however, the applicant should be required to provide a detailed account of the transformation procedure. Based on the method used to construct the GMO the possibilities for the introduction of additional DNA elements, e.g. from impurities during DNA preparation or by the specific transformation system used, should be discussed by the notifier. The assessment of the GMP for extraneous sequences introduced by the transformation method should be based on this discussion. The notifier should further refer to the general possibilities for the unintentional introduction of DNA-aberrations by the specific transformation method used.
C. INFORMATION RELATING TO THE GENETIC MODIFICATION	Line 791: The draft document includes an addition that the requirements for molecular data (which are the same according to Directive 2001/18/EC and Reg. (EC) 1829/2003) "may depend on the scope of the application". The addition does not contain any specific information which differences in molecular data are subject to a specific scope of a notification. Therefore, this addition unnecessarily adds ambiguity to the guidance. Furthermore, a complete set of molecular data is considered necessary for an adequate risk assessment regardless of the scope of the notification. The addition should thus be omitted.
4.1.3 Exposure assessment	Line 677 onwards: It is written in the text, "The aim of the exposure assessment is the quantitative estimation of the likely exposure of humans and animals to GM plant derived products (e.g. food/feed, pollen, new constituents)". Due to the fact that chapter II is dealing with the (viable) whole GMO, a limitation to "GM plant derived products" is not appropriate.

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3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Line 619 onwards: It should be stated that also additional and specific requirements may be necessary in case of a specific scope (e.g. import only). The tiered risk assessment approach presented here is not considered as relevant for GMO risk assessment (line 623). An exposure assessment cannot be the last step in the ERA, but must represent the basis of the ERA when a GMO is intended to be placed on the market. An exposure assessment will identify those organisms most likely to be exposed and the likely exposure levels (i.e. expression levels) under relevant environmental conditions, and thus, also influence the further testing strategy to be chosen. Thus, exposure assessment is a starting point in the ERA process (see Hilbeck & Andow 2004, Hilbeck et al. 2006). The tiered approach should, thus, be understood in the sense of a "hierarchical" testing of effects commencing with simple tests on few species and advancing through a tiered sequence of tests which increase in complexity, sophistication, cost and duration (Cairns 1981 cited in Andow & Hilbeck 2004). In this context, it is unacceptable that with this approach no field studies would be required if no risks in lab tests have been identified. Therefore, lines 2056-2059 of Chapter III, D.9.5. "Interactions of the GMP with non-target organisms" should be deleted as this approach of the ERA does not correspond to current scientific standards. [Andow, D. A. & A. Hilbeck (2004). Science-based risk assessment for nontarget effects of transgenic crops. BioScience 54 (7). 637-649. Hilbeck A., Fontes E. & D. A. Andow (eds) (2006). Environmental Risk Assessment of genetically modified organisms. Volume 2: A case study of Bt cotton in brazil. CAB International, Wallingford, UK. Hilbeck A. & D. A. Andow (eds) (2004). Environmental Risk Assessment of genetically modified organisms. Volume 1: A case study of Bt maize in Kenya. CAB International, Wallingford, UK.]
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Line 617: The assessment of "environmental damage" as defined by Directive 2004/35/EC and referred to in the guidance document at hand, is not the aim of the environmental risk assessment. According to Directive 2001/18/EC (Annex II), "The objective of an ERA is, on a case by case basis, to identify and evaluate potential adverse effects of the GMO, either direct or indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have". Potential adverse effects certainly differ from environmental damages as specified by "EC, 2004". In addition, there is currently no consensus among stakeholders which respect to environmental damages of GMOs. Thus, the wording should stick to the original intention of Directive 2001/18/EC and avoid the term "environmental damage".
2.3 Intended and unintended effects	Line 598 onwards: Relating to unintended effects the draft document should include all the information that is provided in the chapters 14 to 17 of the Codex Alimentarius Guideline (Codex Alimentarius, 2003). For instance, it should be referred that "unintended effects in recombinant-DNA plants may arise through subsequent conventional breeding of the recombinant-DNA plant", that "the random insertion of DNA sequences may cause silencing, activation of genes or modification in the expression", and that "the assessment for unintended effects takes into account the agronomic/phenotypic characteristics of the plant". [Codex Alimentarius, 2003. Codex principles and guidelines on foods derived from biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards

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	Programme, Food and Agriculture Organisation: Rome. ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf (URL as of date 17-09-2008)]
2.2 Concept of substantial equivalence or comparative safety assessment	Line 580-584: A "comprehensive safety and nutritional assessment of the GM crop derived food/feed per se" should always be carried out, and not only in cases where an appropriate comparator is lacking (see also lines 968-970).
7.1.1. Choice of the comparator	Line 580-584: A "comprehensive safety and nutritional assessment of the GM crop derived food/feed per se" should always be carried out, and not only in cases where an appropriate comparator is lacking (see also lines 968-970).
2. COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS	Line 543-550: Direct comparison of a GM plant with the traditional species has its limits as the general ecological behavior is deduced from a few selected morphological traits that have been assessed. The underlying "additive concept", as described in the quoted OECD document, is one of the key problems of the guidance document.
4.2 Issues to be considered for the Risk Assessment of GM Plants	Line 720: It is unclear why the paragraph on "intended and unintended effects due to the genetic transformation event" has been omitted. This is not regarded as justified, as detection of intended and unintended effects plays a key role during the risk assessment process. Since the importance of the identification of differences (intended and unintended) is emphasised in the draft document on several occasions (e.g. line 549, line 951), we highly recommend having this paragraph included in the text.
1. INTRODUCTION	The EFSA GMO Panel considers monitoring as risk management issue (see lines 506, 2709, 2908-2909). As this guidance is not supposed to consider issues related to risk management (line 283), it is unclear why guidance on monitoring is included in this document at all. This represents an inconsistent approach. In view of the aim of the European Commission to enact a legal framework containing this guidance, it is suggested to exclude the chapter on the Environmental Monitoring plan (chapter 11). An inclusion of this chapter may be possible at a later stage, after sufficient consultation of member states and consideration of member states' opinions on this topic have taken place.
1. SCOPE OF THE DOCUMENT	The EFSA GMO Panel considers monitoring as risk management issue (see lines 506, 2709, 2908-2909). As this guidance is not supposed to consider issues related to risk management (line 283), it is unclear why guidance on monitoring is included in this document at all. This represents an inconsistent approach. In view of the aim of the European Commission to enact a legal framework containing this guidance, it is suggested to exclude the chapter on the Environmental Monitoring plan (chapter 11). An inclusion of this chapter may be possible at a later stage, after sufficient consultation of member states and consideration of member states' opinions on this topic have taken place.
2. HOW TO CARRY OUT THE RISK CHARACTERISATION	The EFSA GMO Panel considers monitoring as risk management issue (see lines 506, 2709, 2908-2909). As this guidance is not supposed to consider issues related to risk management (line 283), it is unclear why guidance on monitoring is included in this document at all. This represents an inconsistent approach.

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	In view of the aim of the European Commission to enact a legal framework containing this guidance, it is suggested to exclude the chapter on the Environmental Monitoring plan (chapter 11). An inclusion of this chapter may be possible at a later stage, after sufficient consultation of member states and consideration of member states' opinions on this topic have taken place.
4. THE RESULT OF RISK CHARACTERISATION	The EFSA GMO Panel considers monitoring as risk management issue (see lines 506, 2709, 2908-2909). As this guidance is not supposed to consider issues related to risk management (line 283), it is unclear why guidance on monitoring is included in this document at all. This represents an inconsistent approach. In view of the aim of the European Commission to enact a legal framework containing this guidance, it is suggested to exclude the chapter on the Environmental Monitoring plan (chapter 11). An inclusion of this chapter may be possible at a later stage, after sufficient consultation of member states and consideration of member states' opinions on this topic have taken place.
1. SCOPE OF THE DOCUMENT	In general, the same criticism applies as has already been outlined during the commentary period of the former Guidance Document. These major general shortcomings are: • The "additive concept" (protein + plant) focusing on the expressed transgene product (e.g. in toxicological testing) and neglecting the whole plant, and thus plant-toxin interactions as well as unintended effects on the plant, in particular, also in the case of stacked events. • Conclusions on the safety of the GMP based almost solely on the concept of familiarity and substantial equivalence, and a lack of specific data and specific testing by the notifier. This 'indirect evidence' also overemphasizes the significance of these concepts and contradicts the statement that they should be used as a "starting point" in the ERA (see line 548). • The lack of specification of data requirements (including methods, parameters) and the lack of decision trees for specific testing requirements. • The lack of a clear guidance on how the results of the exposure assessment influence the choice and design of the effects assessment (i.e. for toxicological studies). • The lack of clear guidance on how to address uncertainty in risk assessment (guidance on how to document and consider different levels of uncertainty) and how this uncertainty is mirrored in the monitoring plan. This is considered insufficient because uncertainties according to the current state of knowledge and the limited scope of the ERA should be specifically addressed according to the draft guidance document (lines 643-648). • The lack of consideration of protected species and habitats in the risk assessment. • The case-by-case approach should be followed also for stacked event GMPs. Each stacked event must be subject to an individual risk assessment which cannot be replaced by the risk assessment of the single event stack as foreseen in the current document. Also in the case of stacked events, at least one control line has to be non-GM with a comparable genetic background.
III. INFORMATION	In general, the same criticism applies as has already been outlined during the commentary period of the former Guidance Document. These major general shortcomings are:

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REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED	• The "additive concept" (protein + plant) focusing on the expressed transgene product (e.g. in toxicological testing) and neglecting the whole plant, and thus plant-toxin interactions as well as unintended effects on the plant, in particular, also in the case of stacked events. • Conclusions on the safety of the GMP based almost solely on the concept of familiarity and substantial equivalence, and a lack of specific data and specific testing by the notifier. This 'indirect evidence' also overemphasizes the significance of these concepts and contradicts the statement that they should be used as a "starting point" in the ERA (see line 548). • The lack of specification of data requirements (including methods, parameters) and the lack of decision trees for specific testing requirements. • The lack of a clear guidance on how the results of the exposure assessment influence the choice and design of the effects assessment (i.e. for toxicological studies). • The lack of clear guidance on how to address uncertainty in risk assessment (guidance on how to document and consider different levels of uncertainty) and how this uncertainty is mirrored in the monitoring plan. This is considered insufficient because uncertainties according to the current state of knowledge and the limited scope of the ERA should be specifically addressed according to the draft guidance document (lines 643-648). • The lack of consideration of protected species and habitats in the risk assessment. • The case-by-case approach should be followed also for stacked event GMPs. Each stacked event must be subject to an individual risk assessment which cannot be replaced by the risk assessment of the single event stack as foreseen in the current document. Also in the case of stacked events, at least one control line has to be non-GM with a comparable genetic background.
II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS	In general, the same criticism applies as has already been outlined during the commentary period of the former Guidance Document. These major general shortcomings are: • The "additive concept" (protein + plant) focusing on the expressed transgene product (e.g. in toxicological testing) and neglecting the whole plant, and thus plant-toxin interactions as well as unintended effects on the plant, in particular, also in the case of stacked events. • Conclusions on the safety of the GMP based almost solely on the concept of familiarity and substantial equivalence, and a lack of specific data and specific testing by the notifier. This 'indirect evidence' also overemphasizes the significance of these concepts and contradicts the statement that they should be used as a "starting point" in the ERA (see line 548). • The lack of specification of data requirements (including methods, parameters) and the lack of decision trees for specific testing requirements. • The lack of a clear guidance on how the results of the exposure assessment influence the choice and design of the effects assessment (i.e. for toxicological studies).

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	• The lack of clear guidance on how to address uncertainty in risk assessment (guidance on how to document and consider different levels of uncertainty) and how this uncertainty is mirrored in the monitoring plan. This is considered insufficient because uncertainties according to the current state of knowledge and the limited scope of the ERA should be specifically addressed according to the draft guidance document (lines 643-648). • The lack of consideration of protected species and habitats in the risk assessment. • The case-by-case approach should be followed also for stacked event GMPs. Each stacked event must be subject to an individual risk assessment which cannot be replaced by the risk assessment of the single event stack as foreseen in the current document. Also in the case of stacked events, at least one control line has to be non-GM with a comparable genetic background.
IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL IMPACT	In general, the same criticism applies as has already been outlined during the commentary period of the former Guidance Document. These major general shortcomings are: • The "additive concept" (protein + plant) focusing on the expressed transgene product (e.g. in toxicological testing) and neglecting the whole plant, and thus plant-toxin interactions as well as unintended effects on the plant, in particular, also in the case of stacked events. • Conclusions on the safety of the GMP based almost solely on the concept of familiarity and substantial equivalence, and a lack of specific data and specific testing by the notifier. This ‘indirect evidence’ also overemphasizes the significance of these concepts and contradicts the statement that they should be used as a "starting point" in the ERA (see line 548). • The lack of specification of data requirements (including methods, parameters) and the lack of decision trees for specific testing requirements. • The lack of a clear guidance on how the results of the exposure assessment influence the choice and design of the effects assessment (i.e. for toxicological studies). • The lack of clear guidance on how to address uncertainty in risk assessment (guidance on how to document and consider different levels of uncertainty) and how this uncertainty is mirrored in the monitoring plan. This is considered insufficient because uncertainties according to the current state of knowledge and the limited scope of the ERA should be specifically addressed according to the draft guidance document (lines 643-648). • The lack of consideration of protected species and habitats in the risk assessment. • The case-by-case approach should be followed also for stacked event GMPs. Each stacked event must be subject to an individual risk assessment which cannot be replaced by the risk assessment of the single event stack as foreseen in the current document. Also in the case of stacked events, at least one control line has to be non-GM with a comparable genetic background.
1. SCOPE OF THE	The Draft EFSA Guidance Document at hand builds upon previous experience gained with the first edition of the EFSA guidance Document (EFSA

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DOCUMENT	2006). Although the document at hand contains some improvements, in particular, with respect to the food and feed safety assessments, it still contains a number of shortcomings and gaps in important components of the risk assessment. In particular, the guidance on the field trials for generation of data for plant composition only addresses food/feed safety aspects, but not environmental aspects. Furthermore, important parts of the document have been removed without adequate substitution (e.g. former chapter II 5, e.g. with respect to the careful use of ARM genes and the aim to reduce environmental exposure and the potential risks from the transgenes and their products), and some inconsistencies with the current legal framework are evident and need revision. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]
III. INFORMATION REQUIRED IN APPLICATIONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED	The Draft EFSA Guidance Document at hand builds upon previous experience gained with the first edition of the EFSA guidance Document (EFSA 2006). Although the document at hand contains some improvements, in particular, with respect to the food and feed safety assessments, it still contains a number of shortcomings and gaps in important components of the risk assessment. In particular, the guidance on the field trials for generation of data for plant composition only addresses food/feed safety aspects, but not environmental aspects. Furthermore, important parts of the document have been removed without adequate substitution (e.g. former chapter II 5, e.g. with respect to the careful use of ARM genes and the aim to reduce environmental exposure and the potential risks from the transgenes and their products), and some inconsistencies with the current legal framework are evident and need revision. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]
II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS	The Draft EFSA Guidance Document at hand builds upon previous experience gained with the first edition of the EFSA guidance Document (EFSA 2006). Although the document at hand contains some improvements, in particular, with respect to the food and feed safety assessments, it still contains a number of shortcomings and gaps in important components of the risk assessment. In particular, the guidance on the field trials for generation of data for plant composition only addresses food/feed safety aspects, but not environmental aspects. Furthermore, important parts of the document have been removed without adequate substitution (e.g. former chapter II 5, e.g. with respect to the careful use of ARM genes and the aim to reduce environmental exposure and the potential risks from the transgenes and their products), and some inconsistencies with the current legal framework are evident and need revision. [EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]
IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL	The Draft EFSA Guidance Document at hand builds upon previous experience gained with the first edition of the EFSA guidance Document (EFSA 2006). Although the document at hand contains some improvements, in particular, with respect to the food and feed safety assessments, it still contains a number of shortcomings and gaps in important components of the risk assessment. In particular, the guidance on the field trials for generation of data for plant composition only addresses food/feed safety aspects, but not environmental aspects. Furthermore, important parts of the document have been removed without adequate substitution (e.g. former chapter II 5, e.g. with respect to the careful use of ARM genes and the aim to reduce environmental exposure and the potential risks from the transgenes and their products), and some inconsistencies with the current legal framework are evident and need revision.

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IMPACT	[EFSA, 2006. Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (Question No EFSA-Q-2003-005). The EFSA Journal (2006) 99, 1—100.]
7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed	I refer you to a report that collates almost all of the animal feeding trials that have found negative effects from the consumption of GMOs. In the majority of cases, these findings were not followed up and taken seriously in the way that should be mandatory for a precautionary approach to public health. Please confirm by email that the important evidence contained in this report will be taken into account, as a consultation such as this would be worthless if such important evidence were overlooked simply because it did not fit in a pre-designed response form. http://www.soilassociation.org/Web/SA/saweb.nsf/cfff6730b881e40e80256a6a002a765c/62b3b08dfb6cdaea80256a9500473789/$FILE/gm_health_effects.pdf
7.2.5. Toxicological testing of the whole GM food/feed	It is crucial that EFSA requires that all deaths are included among the negative results and that any death rate over the level in the control non-GM group results in approval being withheld. This should not need stating but, incredibly, death is not being treated as if it were a serious health problem at the moment. We are thinking here of the fact that the GM Flavr Savr tomato was approved by the UK even though seven of the 40 rats died in the two-week company animal feeding trial. Also, it appears some biotechnology companies have been excluding dead animals from the official results of their trials, on the grounds that the effects of the GMO could not be assessed in dead animals. Quality assurance will be a vital aspect of the reliability and credibility of the use of animal feeding trials in EFSA"s risk assessment process. Currently, there is good evidence of widespread use of sub-standard trials by companies - this is one of the main reasons for the lack of trust in current procedures. We propose fours essential requirements. First, all animal feeding trials must be carried out by Government accredited independent laboratories. The fact that the companies have been doing most of the research and that there has been widespread abuse of scientific methodology and reporting to manipulate the approval process, needs to be addressed. As trials in other sectors, such as the generation of safety data for the pesticide industry, are carried by independent laboratories but this is not the case for GMOs, has only aggravated the lack of trust. Second, there must be a requirement that the full results of ALL animal feeding trials are submitted to the regulatory authorities. The possibility of selective disclosure of only "good" results must be prevented. Thirdly, there needs to be an independent "peer review" process where the quality of the trial design and report write-up are scrutinised, and uncertainties clarified. Only once reports pass this stage (and there must be no restrictive time-limit), should they be passed to the next stage, for safety assessment. This is important if the process is to be trusted as truly "science-based, and particularly if company data is to be used. The fact that many of the current studies are of such poor quality and have not undergone a peer review process, means the submissions are not generally considered as proper reliable scientific evidence like scientific papers scientific journals. Fourthly, all results and full reports must be made available to the public. This is essential if EFSA are to secure public trust. GMOs should not be considered safe if the level of any negative effects found would be considered unacceptable if replicated at a population level. Although this is obvious, it is clear that many GM animal feeding trials are not being carried out according to acceptable standards at the moment, and sample sizes are usually so small that important negative effects that would be a public disastor is replicated in

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	the population and would result in a rejection of the product in other sectors are being routinely dismissed as "statistically insignificant". This must be addressed ib this report. One general principle, is that the assessment must be truly precautionary, with public interest clearly prioritised over commercial or political interests. This means that where there is ANY doubt or significant difference of opinion, then the benefit of the doubt must never be given to the GMO/company but to public safety, and approval must be withheld. This would be a large change of strategy from the current process but is essential if EFSA is to achieve its primary role of ensuring safety of GMOs and also of gaining public trust.It is highly important that post-translational effects are assessed, such as those shown by the GM pea study in Australia. This are not currently addressed by the current limited analytical approach - nor is the instability of GMOs.
7.2.5. Toxicological testing of the whole GM food/feed	It is unscientific and biased to indicate that "modifications targeted at specific alterations of metabolic pathways" lead to "improved nutritional and/or health characteristics. Such statements should not be made in an objective paper by European scientific advisers. It is one thing for such targetted modification to be done with the purpose of improving a specific nutritional/health quality, and quite another to assume or suggest that such changes result in a general improvement in the nutritional and/or health chacteristics (as this text suggests) without acknowledging the fact that "normal" nutritional qualties may be lost or impaired due to the wide range of genetic mutatations and biochemical changes routinely found in GMOs. As this is one of the main health and safety concerns over GMOS and the area where it is being felt that EFSA is not taking a sufficiently objective and responsible approach, it is important that statements like this are avoided.
2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL	Line 333: The statement, "products can only be authorised by risk managers once the applicant has adequately demonstrated that the product satisfies these requirements" is not following the Regulation (EC) 1829/2003 and seems to be misleading, since authorisation is given through a complex process involving different bodies of the European Commission and European Union Member States. As the term "risk managers" is not used within the entire Regulation (EC) 1829/2003, to comply with the definitions and specifications of the legal framework, it should also not be used in this context.
V. REFERENCES	Lines 3103 ff.: The full details of the EFSA documents cited in lines 2354, 2355 and 2400 (EFSA 2004c, 20004d, 2005a, 2005b, 2005c, 2005d, 2005e) are missing from the bibliography. Lines 3113 ff.: The following document must be cited here: EFSA, 2008. Safety and nutritional assessment of GM derived food and feed. The role of animal feeding trials. Food and Chemical Tox. 46, 2-70.
3. ISSUES TO BE CONSIDERED FOR RISK CHARACTERISATION	Comparative analysis Lines 2832 – 2835: Another important issue to be addressed is whether unintended effects of potential significance have been missed. Where the occurrence of unintended effects cannot be excluded, strategies to assess the potential human/animal health and environmental implications should be explained. Through the studies recommended in the updated guidance it is ensured that unintended effects which could have an impact on the health of humans and animals, or on the environment, can be detected with a high degree of certainty. In general, however, it is difficult to conceive how it could be decided whether or not

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	unintended effects of the genetic modification have been missed. From a scientific viewpoint it will never be possible to completely rule out the occurrence of unintended effects. Therefore, the paragraph should be deleted. Food/feed safety in relation to intake Lines 2851 – 2852: Data on the characteristics of the compounds including potential biological effects in humans and animals, and effects in the environment should be considered. The words “and effects in the environment” should be deleted, since this section of the guidance document deals with food and feed safety and effects in the environment are addressed in the next section.
7.7. Post-market monitoring of GM food/feed	Lines 1867 – 1899: Given the practical difficulties associated with the performance of post-market monitoring (PMM), the opinion given in the draft EFSA guidance document requires PMM only in special cases. At another point in this section it is explained that a pre-market risk assessment cannot fully reflect the diversity of the population groups that will consume the GM product and, therefore, there is a possibility that unanticipated side effects may occur in certain members of the population. Both statements are true as such. But since one statement stresses the importance of carrying out PMM, while the other statement recommends the performance of PMM in special cases only, it would be advisable to explain more clearly why, in many instances, it is PMM is not called for. Furthermore, it would be favourable if this section could contain more details as a guideline for performing PMM.
7.6. Conclusion of the toxicological/nutritional and allergenicity assessment	Lines 1836 – 1837 This conclusion applies only to GM food/feed from plants with input traits. It should be pointed out that this does not necessarily apply to altered composition (output traits).
7.4. Nutritional assessment of GM food/feed	Lines 1686 ff.: According to section III.D.7.2.5 in relation to herbicide treatment, the criteria to be met by the test materials should be specified for feeding studies with herbicide-tolerant plants.
7.3.2. Assessment of allergenicity of the whole GM plant or crop	Lines 1673 ff.: A comparison of the allergenic repertoire of GM and non-GM organisms basically makes sense. However, few comments are made on how this should be done. At the very least it should be pointed out that for these studies the natural biovariability must be taken into account (e.g. protein expression effects due to growing conditions, climate, supply of nutrients, etc.). This means that to obtain statistically valid evidence a large number of samples must be tested. The term “allergenic repertoire” is so broad, that on the basis of this statement extremely labour-intensive testing could be demanded. Another question arises in relation to this: For example, is it really relevant for a person with a soy allergy if the allergen beta-conglycinin is expressed at a 30% higher level in a genetically modified soybean? This person would have to avoid the allergenic food in any case. A really critical case, on the other hand, would be if a protein from a strongly allergenic protein family (e.g. a 2S albumin) that is not normally expressed in the edible part of the food plant becomes expressed in the genetically modified plant. This would really give cause for concern. The suggested approach would perhaps not address these issues, because to date a non-expressed protein from an allergenic protein family has never triggered an allergy, since, of course, such proteins were not consumed. This theoretical

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	situation is not covered in the guidance document.
7.3.2. Assessment of allergenicity of the whole GM plant or crop	Lines 1607 – 1622: The statements on how sequence comparisons with known allergens are to be conducted are extremely vague. A level of 35% identity over a segment of 80 amino acid residues is widely accepted and has proven to be very effective. If, in addition, a sequence comparison of smaller peptide fragments is required (as hitherto designated by the EFSA), then the size of these fragments should be clearly stipulated: Footnote 10 does go into this (6 or 8 amino acids), but in light of the fact that in the past this point has repeatedly led to disagreements, the EFSA GMO Panel should be asked to state its position in more detail. Lines 1653 ff: The value of "Targeted Serum Screening" (recommended in WHO 2001) was, after lengthy debate, considered critical in the subsequent Codex document, since the clinical relevance of potentially observed low-affinity cross-reactions is unclear. It was concluded that the evidence provided by these tests must be validated. In this regard, the EFSA should be asked to at least conduct a critical evaluation. Line 1662: The "search for T cell epitopes" mentioned here is, in principle, a good idea that has already been brought into the discussion on various occasions. The problem is that the clinical relevance of cross-reactive T cells for food allergies is, to a large extent, undefined. It should be made insistently clear that interesting approaches do exist. However, these still need to undergo comprehensive analysis and validation before they can be applied in the assessment process.
7.2.5. Toxicological testing of the whole GM food/feed	Lines 1442 – 1456: In section III.D.7.1.2 it was established that in the case of herbicide-tolerant plants, three test materials should be compared: 1) genetically modified plants that have been treated with the complementary herbicide, 2) control plants/crops that have been treated with conventional herbicides and 3) genetically modified plants/crops that have been treated with the same conventional herbicide as the control plants/crops. The description of the 90-day toxicity study in rodents lacks the relevant information on whether the animal feed should contain all three test materials. Accordingly, in the case of herbicide-tolerant GM plants, the XXX recommends that the criteria to be met by the test materials be specified. Line 1454: The formulation "remaining uncertainties" is too vague. Either the conditions under which the results of an analysis conclude that "uncertainties" (in contrast to "indications") with regard to unintended effects are present should be defined, or the words "or remaining uncertainties" should be deleted. Lines 1474 – 1484 and lines 1554 – 1564: This paragraph appears in identical form in two places in the text and should thus be deleted from one of the two places. Furthermore, the statement that a 90-day rat feeding study is generally required in cases where multiple genes were transferred is not comprehensible. Rather a case-specific decision is also indicated here, depending on the results of the molecular analysis, the comparative compositional analysis, the knowledge about the mode of action of the expressed proteins, the existence of pathways for potential interactions and, where applicable, the results of toxicity tests in which the respective proteins were administered orally. Lines 1462-1463:

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	It should be pointed out that the requirement that "The highest dose level should be the maximum achievable without causing nutritional imbalance" for genetically modified plants with output traits and/or in the case of an absence of substantial equivalence to non-GM plants can only be realized after performing the appropriate dose-response study. Only after this, and subject to the bioavailability of the relevant nutrients, is a balanced dosage design (also for rats) possible. Lines 1515 – 1522: With regard to the evaluation of effects observed in animal feeding studies, it is required that the evaluation be carried out by experts. It is not clearly stated whether the evaluation is to be conducted by the applicant or by independent experts. III.D.7.3 Allergenicity Lines 1573 ff. The terms used do not conform to standard textbook terminology. When comparing protein sequences one may find “amino acid sequence identity” and “amino acid similarity”. “Homology” relates to either genes or structures, but not to protein sequences.
7.2.2. Toxicological testing of newly expressed proteins	Lines 1364 – 1414: The genetic modification may result in the parallel expression of two or more proteins in the GM plant. This section contains no information on whether, or in which cases, these proteins must be tested in combination. A corresponding reference to the combined administration of proteins is found in a later section (III.D.7.2.5: lines 1474 – 1484 and 1554 – 1564) which deals with the toxicological testing of the whole GM food/feed. Since section D.7.2.2 relates to the toxicological testing of newly expressed proteins, it is advisable either to present the pertinent information here, or to insert a reference to the above-mentioned text passage. Lines 1368 – 1370: It is stated that specific toxicity testing may not be required if, according to long-standing experience, both the plant and the newly expressed protein are considered safe for consumption by humans and animals. In this context it would be desirable to specify whether the amino acid sequence of the protein expressed in the genetically modified plant has to be 100% identical to the sequence of the approved protein. Following that the question arises of whether a bioinformatics-based approach to the evaluation of the protein is sufficient when differences in the amino acid sequence are observed. Lines 1375 – 1378: The characteristics that must be identified to establish equivalence between a plant-produced protein and a bacterially synthesized protein are cited by means of giving examples. In this regard the specification of minimum mandatory requirements for the determining characteristics is recommended, rather than the provision of examples.
7.2.1. Standardized Guidelines for Toxicity Tests	Lines 1357 – 1359: The fact that the document advises against the performance of acute toxicity tests with the newly synthesised protein, on the grounds that such tests are of little significance for the risk assessment of repeated consumption of the GMO when used as food/feed, should be critically assessed. Sub-acute and sub-chronic toxicity studies with rodents are suggested in order to achieve relevant exposure conditions. Although this statement is true, thought should be given to the fact that

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	proteins, in particular, undergo a rapid break-down in the gastrointestinal system and therefore accumulation in animals and humans is generally excluded. Hence potential safety risks from the effects of bioaccumulation can be virtually disregarded. It is suggested that the decision on the necessity for long-term toxicity studies should depend on the outcome of the assessment of other characteristics of the newly synthesised protein, e.g. stability tests (SGF, SIF, thermostability), the amino acid sequence identity comparison and possible indications of alterations in the composition or in the content of the constituents. Accordingly, when using a holistic approach it may suffice to perform an acute toxicity study rather than a repeated-dose 28-day toxicity study, as long as additional significant data are presented.
7.2. Toxicology	Lines 1323 – 1327: This passage states that the toxicological analysis should be performed in order to demonstrate that unintended effects of the genetic modification(s) that/which have been identified, or that/which may be assumed to have occurred based on the preceding comparative molecular, compositional or phenotypic analyses, have no negative effects on animal or human health. Testing of individual components and/or of the whole GM food/feed can be considered for this purpose. As previously commented, the document does not go into enough detail on when differences with regard to one or more parameters should be classified as an unintended effect of the genetic modification. In this context the wording "unintended effect(s) of the genetic modification(s) that may be assumed to have occurred" is an unfortunate choice of phrase, since it could be deduced that alone the assumption that an unintended effect exists would be sufficient reason to demand the performance of extensive toxicological studies. Since statistically significant differences resulting from the natural variance can be falsely interpreted as "unintended effects", studies on the whole GM (plant-derived) food/feed, for instance in the form of a 90-day oral toxicity study in rats, could be demanded, although in reality no unexpected effects of the genetic modification exist and/or the effects that do occur are not biologically relevant.
7.1.7. Conclusion of the comparative analysis	rther consideration in the risk assessment process or if the difference and/or lack of equivalence does not raise safety concerns." (Underlining added). In the opinion of the XXX this step in the evaluation process is essential, since it can be expected that according to the methods described in section III.D.7.1.2 it is probable that non-equivalences will be observed between individual parameters of the GMO and its comparator which are neither attributable to unintended effects of the transformation (genetic modification) nor do they raise safety concerns. This should be presented clearly and consistently in the document in order to avoid varying interpretations of the document and, in some cases, unnecessary animal trials. In detail, revision is required in the following points: Lines 1286 – 1290: These two paragraphs explain that for the interpretation of the results of the comparative analysis it should be clearly stated whether the GM plant(s) or product(s) differs from its non-GM comparator with respect to composition, agronomic and phenotypic characteristics (first point), or whether the GM plant is equivalent to its non-GM comparator with respect to composition (second point). From the text it is not clear why these two points are addressed in two discrete paragraphs, or to what extent the description of these two points should differ in the application documents. In connection with the newly drafted section III.D.7.1.2, it is essential that a clear definition of the terms "difference" and "equivalence" or "non-equivalence" be included in the document.

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	Lines 1291 – 1298: This paragraph deals with characteristics in which the GM plant or its product(s) are not equivalent to the non-GM comparator, and which should be considered as unintended effects. It is not clear whether from the point of view of the EFSA GMO Panel an unintended effect already exists when non-equivalence according to III.D.7.1.2 is detected in relation to just one parameter (see comments above on the necessity to define the terms “difference” and “equivalence” or “non-equivalence) nor is it clear how cases of “probable non-equivalence” and “probable equivalence” are to be dealt with. The question arises as to when exactly a difference/lack of equivalence should be classified as an unintended effect requiring further testing, for example, a 90-day oral toxicity study in rats. In the opinion of the XXX it must be clearly stated that when differences are detected it will be decided on the basis of a subsequent evaluation whether additional assessment is required. Similarly it should be emphasised that additional toxicological testing on food/feed (animal studies) is not automatically required when differences based on the methodology described in the newly drafted section III.D.7.1.2 are observed. Reference is made to lines 2793 – 2831 of the updated draft. This reference seems appropriate in this section of the document. Lines 1299 – 1300: This sentence points out that the methods specified in section III.D.7.2.1 are also suitable for confirming the presence of intended effects. In the opinion of the XXX this reference can be omitted here. A similar sentence concerning GM plants in which the genetic modification is targeted at an alteration in the composition appears in lines 1230 – 1231. Remarks relating to intended effects which involve alterations in the phenotypic or agronomic characteristics could be introduced in section III.D.7.1.5.
7.1.5. Comparative analysis of agronomic and phenotypic characteristics	Lines 1256 – 1248: Here it is explained that possible differences in the phenotypic and agronomic characteristics of stacked events must be assessed in field trials over a minimum period of one year. Since in the newly drafted section III.D.7.1.2 the requirement for field trials with single events with an equivalent number of experimental sites is also just one year, the requirements for stacked events should be described more precisely in this passage.
7.1.4. Comparative analysis of composition	Line 1196: It is stated that the compositional analysis should include both an appropriate selection of chemical compound as well as the newly expressed proteins. The testing of newly expressed proteins is not part of the comparative analysis of composition and should not be specified here. The testing of newly expressed protein is dealt with in sections III.D.3 and III.D.7.2.
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	conducted in Europe? If so, must additional trials be carried out in the exporting country, since its production regulations may differ from those in Europe? In practice, clear minimum requirements, to which adherence can be conclusively verified, are more effective than open-ended targets. Criteria for the respective environments (= limited number of climatic regions) should be set. Lines 1030 – 1031: The clear provisions on the number of experimental sites and years required in lines 1050 – 1052 are relativised in lines 1030 – 1031. These statements should agree and be brought together in one passage of the document. Lines 1056 – 1059:

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	The requirements for describing field trials should be extended to include the parameters fertilisation, growth (plant density) and plant health/pest infestation. Lines 1074 – 1079: Rather than giving examples of possible data transformations, specific target requirements for the preparation and transformation of the data should be formulated. Whether or not the performance of data transformation is worthwhile can only be derived from the data itself. Line 1128 ff.: Doubts were expressed as to whether the figure pertaining to section 7.1.2 (c) in line 1128 and the legend beneath will be correctly understood and interpreted. It would be helpful to include a practical example for a “statistical analysis for the compositional risk assessment” in the annex.
7.1.1. Choice of the comparator	n that there is no interaction between individual events in a/the lower stack, then the lower stack can substitute as a comparator for the GM parental lines (see also lines 1060 – 1065 of the updated draft of the EFSA guidance document). Lines 984 – 986: This part of the draft refers to the guidance document of the GMO Panel for the risk assessment of genetically modified plants containing stacked transformation events (EFSA 2006). The XXX understands that the aforementioned document will be replaced by the present draft. In that case the 2006 document should not be cited.
7.1. Comparative analysis	Lines 931 – 942: In the introduction to the section on comparative analysis it should be explained that such an analysis can only be performed if appropriate comparators can be identified. In the case that no appropriate comparators can be found, a comprehensive safety assessment should be carried out (compare also lines 956 – 960 of the updated draft of the EFSA guidance document and the corresponding comments of the XXX).
4. Genetic stability of the insert and phenotypic stability of the GM plant	The genetic stability of single events should be demonstrated over five generations (generative and vegetative propagation). The reference to vegetative propagation is unclear. In the potato, for instance, somaclonal mutation may only affect individual buds. In that case the identification of such a mutation is entirely random; since the mutation is not persistently present in all tuber offspring. What constitutes a generation of a vegetative propagation? This remains ambiguous. Does it refer to propagation cycles? It is also not clear why the analysis must be carried out over five (generative) generations. An analysis of the first and the fifth generatively propagated generation would be adequate to detect a genetic instability. No statement is made regarding the origin of the material for the analysis of genetic stability.
3. Information on the expression of the insert	This section was given an introduction, which should clarify the purpose of the requested information on the expression of the insert in the recipient plant. The introduction is divided into two bulleted paragraphs. Point 1: The object of the expression analysis is to demonstrate that the intended effect of the genetic modification has been achieved. The aim is to demonstrate the efficiency of the modification. However this question is not touched on by any of the required studies subsequently listed under (a)-(f). Point 2: A further object of the expression analysis is to examine whether changes in the amino acid sequence of the expressed protein lead to a change in the post-translational modification, or whether the function of the expressed protein is impaired as a result. Similarly, none of the subsequently requested analyses address this point.

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	(c) Potential creation of fusion proteins: The original passage was reduced to a demand for a bioinformatic analysis of existing ORFs. There is, therefore, some redundancy in III.D.2 (f). An expression analysis (Northern) of newly created ORFs is no longer requested at this point. However, in a case-by-case scenario such an analysis can prove quite helpful. (e) Range of protein expression: The requirement to stipulate the range of protein expression in the conditions under which the genetically modified crop is grown, or will be grown, is new. This data should be collected at the same time as the data for the compositional analysis. See criticism brought forward under D7.1.2. Furthermore, it is suggested that RNA studies be carried out. These should be requested on a case-specific basis and can address both the expression of inserted genes and newly generated ORFs. (f) Protein expression in stacked events: The content under this point roughly corresponds to point 3.1.2 of the EFSA document on stacked events. However, only a safety consideration has been requested to date in order to assess whether or not additional safety issues result from the combination of genetic modifications in comparison to single events. Now the provision of data for such an assessment is requested. The scope and type of assessment required, which should be sufficient to document the non-existence of a risk are not defined. The requirement should be reduced to the provision of evidence that the level of expression in stacked events is comparable to that found in single events.
C. INFORMATION RELATING TO THE GENETIC MODIFICATION	Line 771: It is pointed out that the data requirements for molecular characterisation may depend on the scope of the application. This aspect is new. However, the statement is neither substantiated nor elaborated on, so that the exact meaning of this passage of text remains unclear. It should be explained why the data requirements for molecular characterisation may depend on the scope of the application. Examples could be given here such as applications for licences to place on the market of GMO plant-derived products (e.g. sugar derived from genetically modified sugar beets). In the case that no plausible explanation can be given, the part of the sentence containing "…but may depend on the scope of the application" should be deleted. Lines 772–778: The 2006 version of the EFSA guidance document explicitly states that changes in the DNA sequence which have an effect on the amino acid sequence of the gene product should not only be described, they should also be evaluated. This requirement was deleted from the update and should be re-incorporated.
B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS	Lines 735-736: • to evaluate all issues of potential concern, such as the presence of natural toxins, allergens or virulence factors. The words "or virulence factors" should be deleted, since the present document deals exclusively with higher plants and higher plants do not contain virulence factors.
III. INFORMATION REQUIRED IN APPLICATI	Line 704: The word "to" appears twice before "follow". One should be deleted.

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ONS FOR GM PLANTS AND/OR DERIVED FOOD AND FEED	
4.2 Issues to be considered for the Risk Assessment of GM Plants	Lines 694-695: The “compositional characteristics” comprise nutrients as well as anti-nutritive, toxic and allergenic substances (compare section 7.1.4). The additional mention of „nutritional characteristics“ as just one of these substance groups is not justified; the word “nutritional” should therefore be deleted and should instead be inserted into line 695, so that this now reads: “the influence of processing on the nutritional properties of the food or feed”.
4.1.3 Exposure assessment	Lines 651-662: It should be made clear that an assessment of the exposure of humans and animals is only necessary if a possible hazard has been identified in the previous stages of the risk assessment.
2.2 Concept of substantial equivalence or comparative safety assessment	Lines 538-549: The sentence beginning on line 538 with “Application of this concept” should end on line 541 with the words “non-GM comparator”. The sentence beginning on line 546 should be shortened as follows: “The outcome of this comparative analysis will further structure the subsequent assessment procedure, which may include further specific safety and nutritional testing.”
2. COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS	Lines 508-522: The contents of section 2.1 is partially identical to that of section 2.2 (lines 536-551). The sentence beginning on line 518 should therefore be shortened as follows: “This is followed by comparative analyses of the relevant characteristics of the GMO and its non-GM comparator.”
1. INTRODUCTION	Line 497: The reference to the FOSIE publication relates to FOSIE, 2002 (not 2003).
2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL	Line 453 The EC guideline reference in parentheses relates to EC, 2001b (not "c"). Line 454: The exact meaning of “relevant parts” is not clear and should be complemented, for example, by the addition of: “i.e. those parts applicable to the plant and/or genetic modification in question”.
1. SCOPE OF THE DOCUMENT	Line 242: The citation in parentheses refers to EFSA, 2006c.
I. INTRODUCTION	Table of Contents Point II.4.1 is missing between points II.4. and II.4.1.1. Section III.D.12 (“ERA of GM plants containing transformation events combined by conventional breeding”) is missing from the Table of Contents.
I. INTRODUCTION	General comments: The EFSA GMO Panel has submitted a draft for an updated guidance document

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	for the risk assessment of genetically modified plants and derived food and feed. On examining the draft the XXX received the impression that the amendments contained therein, at least to some extent, represent a clear step in the right direction and that the requirements for applicants are clearly stated. Other, significant parts however fall short and should be revised with the aim of improving the level of detail and clarity. The same applies to the many inconsistencies in the text, which arose as a result of the time constraints under which the present draft was produced. The European Commission's Directorate-General for the Environment has asked the EFSA to redraft the "Guidelines on the risk assessment of GMOs" not only with regard to the requirements for the assessment of food and feed safety, but also with regard to the requirements for environmental risk assessment (ERA). The EFSA has estimated a two-year time frame to complete this task. The XXX therefore recommends that the other parts be simultaneously revised according to the given comments so that the guidance document in itself is consistent, and in all parts reflects the current status of development in the risk assessment of genetically modified organisms, food and feed. Irrespective of this, the latest version of the existing document may be used in the interim. The present draft of a revision of the existing "Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO)" in the version dated October 2006 reveals that since the implementation of Regulation (EC) No. 1829/2003 the experiences gathered in the assessment of application documentation have only been partially integrated. Thus, for instance, within section III.D.7.1 ("Comparative analysis"), the subsection III.D.7.1.2 ("Experimental design and statistical analysis of data from field trials for comparative analysis") was essentially rewritten. This subsection now includes concrete and detailed requirements for field trials, with the aid of which the data for an application for placing on the market should be generated. The requirements for the statistical analysis of these data are also given. The XXX welcomes this concretization, which will allow applicants to plan more reliably in the future and at the same time facilitates the assessment of applications. However, other key points in section III.D.7.1 ("Comparative Analysis") were only marginally, or not at all, revised. These points, which are directly related to the field trial requirements in section III.D.7.1.2, are therefore still comparatively vague and, in parts, do not reflect the current state of experiences in risk assessment of genetically modified food and feed. Furthermore, sections of the EFSA guidance document on stacked events from 2006 were inserted into several parts of the present draft without paying sufficient heed to consistency within the document. At one point in the draft (lines 984 – 990), the guidance document on stacked events is referred to in such a way that it is unclear whether the 2006 guidance document is to stay in effect, or whether it is to be replaced by the present draft. The present draft needs to be revised, particularly with regard to the following key points: • Selection of the appropriate comparator (for "single events" and "stacked events"); • methods for the statistical analysis of the data; • connection between "non-equivalence" in the sense of III.D.7.1.2 and "unintended effects", and ambiguity of the terms "probable non-equivalence" and "probable equivalence"; • data requirements in applications for "stacked events" compared to applications for "single events".
4. THE RESULT	9202-2909 The risk characterisation should also evaluate whether adverse effects on human health, animal health or the environment are likely to arise from GM

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OF RISK CHARACTERISATION	food/feed that differs significantly from its conventional comparator.
3. ISSUES TO BE CONSIDERED FOR RISK CHARACTERISATION	2822-2823 Animal studies are likely to be inadequate to characterise the health impacts of GM foods with altered nutritional or medicinal properties: particularly if adverse effects for specific population groups have been identified in clinical trials of the altered constituent nutrient or gene product. More comprehensive human clinical trial data may be needed to define the therapeutic window of nutritionally altered GM food/feed and to meet the Directive's requirement to prevent adverse health effects.
2. HOW TO CARRY OUT THE RISK CHARACTERISATION	2702-2710. Existing human clinical trial data must also be considered for GM foods with altered nutritional or medical properties, and additional data may be required.
2. HOW TO CARRY OUT THE RISK CHARACTERISATION	2702-2710. Existing human clinical trial data must also be considered for GM foods with altered nutritional or medical properties, and additional data may be required.
7.4.2. Nutritional assessment of GM feed	1784-1786 Assessment of the nutritional profile and potential adverse health effects of foods derived from animals fed GM animal feeds with modified nutritional value should be mandatory. In many cases, the purpose of the GM feed will be to alter the nutritional properties of the food intended for human consumption (for example, using omega-3 enhanced GM feed for chickens in order to produce omega-3 eggs). It would be inconsistent to require nutritional assessment of GM foods with altered nutritional properties, but not of foods with the same or similar properties produced via such a two-step process.
7.4.1. Nutritional assessment of GM food	1731-1755 The proposals are inadequate to identify the adverse health effects of altered nutritional properties of GM foods, as noted in comments to section 7.2. Human, not animal, data is needed to address the concern noted in lines 1744-1746 that some populations may benefit whilst others are at risk from the same food. The proposals are also inconsistent with the guidance for nutritionally altered GM feed (lines 1770-1771) which requires livestock feeding studies with the target species, not an alternative species which is likely to metabolise the feed differently. It is hard to understand why this point is recognised as of importance to animal health but not to human health. It should also be noted that some products may have a beneficial effect on certain aspects of health but a harmful effect on others (as, for example, with the cholesterol-lowering effects of plant sterols in margarine, which may simultaneously reduce absorption of fat-soluble vitamins).
7.3. Allergenicity	1590-1592 Although allergenicity does not affect everyone, there is no scientific evidence that it is restricted to persons with a particular genetic make-up. Other factors (such as childhood exposure to infections) may be important.
7.2.5. Toxicological testing of the whole GM food/feed	1449-1457 If the composition of the GM plant is modified substationally, with the intention or expected consequence of altering nutritional or medical properties, human clinical trials of adequate statistical power may be necessary to identify some adverse effects (e.g. carcinogenicity) and effects in vulnerable subpopulations.
7.2. Toxicology	1312-1324 This paragraph is not applicable to foods with altered nutritional or medical properties, because the setting of safe upper limits or no adverse observed effect levels for nutrients or other gene products intended or likely to have effects on human health or metabolism should also take account of data from human clinical trials. Some population groups may also be at higher risk of adverse effects. For example, in the UK, the Food Standards Agency has set a Safe Upper Level for beta-carotene supplementation of 7mg/day for non-smokers and has

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	recommended that smokers or those exposed to asbestos should not take beta-carotene supplements. These recommendations are based on available clinical trial data. For nutritionally-altered GM foods, reliance on animal toxicology studies alone is unlikely to identify all adverse health effects and cannot adequately characterise the potential harms to population subgroups. Further, failure to include clinical trial data is likely to result in inconsistencies between GM crop approvals and the approval of other products with related properties, such as supplements. 1326-1329 As noted above, animal toxicology tests alone are inadequate to demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health, particularly if the GM food/feed is intended or expected to have altered nutritional or medicinal properties. At minimum it will be necessary to consider: a systematic review of existing data from human clinical trials of equivalent or related products (e.g. pharmaceuticals or supplements); data on dose-response and therapeutic window; a systematic review of existing data on potential adverse effects in specific sub-populations (e.g. children, the elderly, smokers), including indirect effects (e.g. masking of vitamin B12 deficiency in the elderly by folic acid supplementation); relevant regulatory decisions and advice, such as upper safe limits and no adverse observed effect levels for vitamins and minerals. To identify and prevent adverse health effects, additional clinical trials of adequate statistical power may also be necessary.
7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed	942-943 Section 7 is inadequate to meet the requirements of Directive 2001/18/EC, because it fails to include sufficient measures to identify hazards from second-generation GM crops (including nutritionally-altered crops) and prevent adverse health effects. In particular, it ignores the results of human clinical trials of supplements and pharmaceuticals, that may indicate long-term adverse effects on health (including carcinogenicity). Human health could be seriously harmed as a result of failure to require this data prior to authorisation. Inconsistencies may also arise with the rules currently under development for the Addition of Vitamins and Minerals and Other Substances to Foods; the Food Supplements Directive; international and national guidelines for food fortification programmes; and the approval process for pharmaceuticals. If GM food or feed is authorised which subsequently fails one of these assessment processes, public trust in the assessment process will inevitably be lost and expensive product recall and decontamination procedures may be required.
3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion	819-826 For gene products intended or expected to have nutritional or medical effects: a systematic review of existing data from human clinical trials of equivalent or related products (e.g. pharmaceuticals or supplements); data on dose-response and therapeutic window; a systematic review of existing data on potential adverse effects in specific sub-populations (e.g. children, the elderly, smokers), including indirect effects (e.g. masking of vitamin B12 deficiency in the elderly by folic acid supplementation).
4.1.4. Risk characterisation	693-697. In the case of nutritionally-altered crops, or crops designed to produce pharmaceutical products, evidence from human clinical trials may be available and/or required to demonstrate safety. Relevant regulatory decisions and advice, such as upper safe limits and no adverse observed effect levels for vitamins and minerals, should also be taken into account.
4.1.2 Hazard characterisation	668-675. The section on hazard charecterisation should note that GM food/feed aimed at altering nutritional or other properties may have a small therapeutic window, in which doses of the nutrient(s) or other plant products are both efficacious and safe. Hazard characterisation will need to identify this window and whether it is appropriate for all population groups: to do this data from human

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	studies may be needed.
4.1.1. Hazard identification	658-666. The section on hazard identification should state that many nutrients may be harmful at high doses, or to particular subpopulations, as may substances not previously contained in food or feed, or normally present at low doses. GM food/feed that is aimed at modifying nutritional quality, or producing new products, such as pharmaceuticals or plastics, therefore require particular attention.
7.3. Allergenicity	Recently, the issue of a possible adjuvant effect of Cry proteins in GMOs (especially maize) has been raised by xxx. It is obvious that maize may be eaten together with other foods containing components to which an immune response may be enhanced due to a possible adjuvant effect of Cry proteins. If Cry proteins act as adjuvants, the effect will be expected to be seen as an increase in allergies to the most commonly allergenic foods rather than to maize. The xxx sees the need for further clarification regarding the possible adjuvant effect of Cry proteins and welcomes a chapter on adjuvance in the allergy section of the EFSA guidance document. The issue of adjuvance should be evaluated on a case by case basis.
OTHER SCIENTIFIC COMMENTS	III.D.9.4. Interactions between the GM plant and target organisms III.D.9.5 Interactions of the GM plant with non-target organisms Line 2049-2075: We recommend to include guidance on which and how many non-target organisms that should be tested. o How many and which indicator species should be tested? The statement in line 2061 “which MAY INCLUDE pollinators, beneficial, predatory and phytophagous species” is very vague. Furthermore, the document only gives general directions on how these tests should be carried out. From this guidance document, the applicant cannot know what is needed, eg in case a toxic protein is expressed in a plant. Would it be impossible to specify data requirements for environmental risk assessment (ERA) for GM plants, e.g. as is done in guidance for ERA of plant protection products, where it is clearly said what categories of non target organisms need to be tested and how this should be done? o Should species selection be based upon species relevant for Europe (i.e. species occurring in the European crop ecosystem)? o A tiered approach is recommended in part 9.5 (line 2056), but is such a tiered approach also required for point 9.8, to test effects on earthworms and other soil organisms? Line 2061: what is meant with “beneficial” as set apart from the other categories? Aren’t pollinators beneficial? Is an insect parasitoid a “predatory species”? III.D.9.6. Effects on human health III.D.9.7. Effects on animal health III.D.9.8 Effects on biogeochemical processes Line 2100-2102: The influence of GM-plants on rhizosphere and soil microbial communities may be difficult to assess, because these bacterial populations are

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	highly variable and a substantial fraction of the strains that are present can not yet be cultured. More detailed instructions on the information that must be provided may be useful. One possibility might be to study a few representative species as indicators, when this is justified by the nature of the gene(s) inserted in the GM plants. Line 2107-2108: "…effects on the recognised soil microbial communities and the associated functional actiities … Although these aspects are clearly relevant, the characterization of microbial communities is not straightforward: how can the applicant address that issue (in practical terms and not in theoretical terms)? III.D.9.9. Impacts of the specific cultivation, management and harvesting techniques Line 2129: "This should include the biodiversity." A definition of biodiversity should be provided: whether it is defined at the level of species, community and/or ecosystem and should include taxa from plant animals and microbes. "Biodiversity within the GM crop": It may be interesting do define more clearly which organisms are involved. Only those species that do not reduce the yield by competing with the crop or damaging it are probably relevant. Editorial comments Line 2143: delete "." after 11
OTHER SCIENTIFIC COMMENTS	III.D.9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification III.D.9.1. Persistence and invasiveness Line 1957-1958: This sentence is not complete. Line 1964-1965: Rephrase the sentence "The information provided ... environment", as this sentence is not clearly formulated. III.D.9.2. Selective advantage or disadvantage Editorial comments Line 1982: references to D 7.2 and 7.4 should be replaced by D 7.1 III.D.9.3. Potential for Gene transfer Line 1988-2001: Bacteria possess very efficient mechanisms (conjugation, transduction, transformation, transposition, …) for the transfer of genetic information, including antibiotics resistance traits within and between bacterial species. On the other hand, gene transfer between plants or plant-derived materials and bacteria, if it occurs, is very hard to demonstrate. In addition, the antibiotic resistance genes present in transgenic plants were often modified to facilitate their expression in plants, with a concomitant decease of the efficiency of expression in bacteria. For these reasons, information on the use of the relevant antibiotics in human and veterinary medicine should be in important factor in the

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	risk evaluation. As a precaution, antibiotics that are currently used and against which resistance is not yet widespread should be avoided. Others are probably not problematic. Line 2021: “In cases where gene transfer cannot be limited between certain adjacent plants, the risk assessment should focus on the consequences of cross-pollination”. This may suggest that, a contrario i.e. when gene transfer can be limited, the consequences of gene transfer are of secondary importance. We propose to pay attention to the risk of gene transfer itself. From a risk management point of view, this distinction is more relevant, however. Line 2035-2037: Please change the sentence as follows: "The applicant should also take into account the information in Sections III D 9.1, 9.2, 9.9 and 10 to evaluate the risks of plant to plant gene transfer". We propose to also refer to 9.9 as introgression will depend on the agricultural management and practices (e.g. weed control).
OTHER SCIENTIFIC COMMENTS	ANNEX IV. There is a lack of correlation between the numbering of the paragraphs in chapter III “Information required in applications for GM plants and/or derived food and feed” and Annex IV point D “information relating to the GM plant” (Line 3785; 3787; 3795;…)
OTHER SCIENTIFIC COMMENTS	III.D.11.4.2 Main elements of general surveillance III.D.11.4.2.1. Existing monitoring systems Line 2396-2399: The inclusion of plans for introduction, marketing, etc. into the monitoring plan, is not only of relevance for studies to be carried out by existing monitoring systems, but also for the GMO-focused monitoring systems. For this reason, we propose to place this paragraph (lines 2396-2399) after line 2393. Editorial comments III.D.11.4.2.1. is not mentioned in table of contents III.D.11.4.2.2. Use of GMO-focussed monitoring systems Editorial comments III.D.11.4.2.2. is not mentioned in table of contents Line 2462: replace "genetically modified" by "GM" III.D.11.4.2.3. Importance of baseline Editorial comments III.D.11.4.2.3. is not mentioned in table of contents III.D.11.4.2.4. Data quality, management and statistical analyses Editorial comments III.D.11.4.2.4. is not mentioned in table of contents III.D.11.4.3 Importance of a baseline

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	III.D.11.4.4. Data quality, management and statistical analyses Editorial comments Line 2536: Ccase-Sspecific Mmonitoring III.D.11.5. Reporting the results of monitoring Editorial comments Line 2565: Cconsent Hholder Line 2568: add "." after Art III.D.11.6. Review and adaptation Editorial comments Line 2610: add "-" between "cost" and "effectiveness"
OTHER SCIENTIFIC COMMENTS	III.D.11.4. General surveillance for unanticipated adverse effects Editorial comments Line 2274: include space between "long-term" and "effects" Line 2276: Ccase-Sspecific Mmonitoring Line 2278: Ccase-Sspecific Mmonitoring Line 2282: replace European Union by "EU" (abbreviation has been used before, e.g. line 2190) Line 2290: add spacing between "adverse." and "The" III.D.11.4.1 Approach and principles of general surveillance Editorial comments Line 2317: add "," after Therefore III.D.11.4.1.1. Approach and principles for GM plants intended for import and processing only Editorial comments III.D.11.4.1.1. is not mentioned in table of contents Line 2330: Guidance dDocument Line 2331: e.g. in italic Line 2339: e.g. in italic Line 2340: GMOs applications Line 2340: include "under" after submitted Line 2342: include "the" before EFSA III.D.11.4.1.2. Approach and principles for GM plants intended for cultivation Line 2357-2366: To our opinion, lines 2357 to 2359 (till "thus") can be removed as they do not add value to the text. This paragraph can start as follows: The focus on general surveillance... We also propose to place this paragraph before the former paragraph (lines 2354-

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	2356) as it is more logic to first address WHAT will be monitored before HOW the monitoring will occur. Editorial comments III.D.11.4.1.2. is not mentioned in table of contents Line 2350: et al. in italic Line 2354: et al. in italic Line 2354: delete "Existing" , as it is also advisable to use other types of surveillance systems (e.g. farmer questionnaires). We propose to refer here to section 11.4.2 for more information. Line 2356: et al. in italic Line 2372: focussed; check focussed throughout text Line 2373: Ccase-Sspecific Mmonitoring Line 2387: include "the" before EFSA
OTHER SCIENTIFIC COMMENTS	III.D.11. Environmental Monitoring Plan III.D.11.1. General Editorial comments Line 2166: Eenvironmental Mmonitoring Line 2168: add "." after Art Line 2170: place) after 17(3)(k) Line 2173: Eenvironmental Mmonitoring Line 2185: "conforming with" shouldn"t this be "conform to"? Line 2205: Guidance Ddocument III.D.11.2. Interplay between environmental risk assessment and monitoring Line 2208: We propose to change the words "foreseen" and "unforeseen" by "anticipated" and "unanticipated", respectively, as these are the terms that are mainly used further down in text. In the same line, we propose to replace "unexpected" (line 2225) and "expected" (line 2251) by respectively "anticipated" and "unanticipated". Line 2227-2231: We propose to change this paragraph as follows: "Directive 2001/18/EC requires that the impacts of any direct effects, e.g. changes in management and cultivations techniques of GM crops, should be addressed by monitoring based on the outcome of the environmental assessement", as - there is a duplication of information in these sentences, namely the example in second sentence corresponds to information in first sentence - beginning the sentence with "The Directive" makes more clear that one is discussing the regulatory framework Line 2235-2239: Is it not more logic to mention first the background environmental data under a) and GM plant-based parameters under b)? Editorial comments Line 2208: Fforeseen Line 2211: delete "unforeseen": saying "unforeseen effects... which were not anticipated" is saying twice the same thing Line 2222: Guidance dDocument Line 2224: (2002/811/EC) Line 2235: include "need to be monitored and" after parameters Line 2243: add "(" before "a)"

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	III.D.11.3. Case-specific GM plant monitoring Line 2274-2278: These lines discuss case-specific monitoring. Therefore, we propose to move these lines to section 11.3 after line 2247. The last sentence can be deleted as it is a duplication of what is mentioned in line 2273.
OTHER SCIENTIFIC COMMENTS	ABOUT EFSA GUIDANCE Editorial comments Line 21-22: remove capital letters from words Risk, Assessment, Derived, Food and Feed. Further down in the text, the Guidance Document names are not mentioned in capital letters. Replace ''Scientific Panel on Genetically Modified Organisms'' by ''GMO Panel'' and ''Genetically Modified Organisms'' by ''GMOs'' Line 24: Ggeneral surveillance Line 25: post-market Line 30: Eenvironmental Line 31: add ''the'' before European Commission TABLE OF CONTENTS Titles of subsections 11.4.1 and 11.4.2 are not mentioned in the table of contents. FOREWORD Line 189: (e.g. seed or other plant-propagating materials): these examples do not clarify specific legislation. Examples should be given here of such specific legislation, such as Regulation on traceability? Line 233-234: Leave out '' - before the date ... 18 April 2004'' , as guidelines have been published after this date. Editorial comments Line 181: replace ''released into'' in ''placed on'' Line 184: add referencee (EC, 2003a) after feed. Be consistent in adding references to Regulations and Directives (e.g. reference them once in each section). They are most of the times referenced, but sometimes not. There is no reference to Regulation (EC) No 258/97 in the reference list. Line 192: place (GMO Panel) after Genetically Modified Organisms on line 191 Line 192: replace Genetically Modified Organisms by GMOs Line 192-193: remove capital letters from words Risk, Assessment, Derived, Food and Feed. Further down in the text, the Guidance Document names are not mentioned in capital letters. Line 194: Ggeneral surveillance Line 200: Eenvironmental Line 201: add ''the'' before European Commission Line 203: Guidance Document (add Document) Line 205: Hans-Joerg should be Hans-Jörg Line 210: ''ad hoc'' should be in italic Line 219: ''ad hoc'' should be in italic Line 220: Boot should be Boet Line 220: Hans Jorg should be Hans-Jörg

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OTHER SCIENTIFIC COMMENTS	General remarks We thank EFSA for updating the guidance document as we consider this reviewing necessary given the experience gained with risk assessment of GM plants and the continuous development in scientific methods. In general, the document has improved. We welcome the introductions explaining briefly the rationale as to why information is asked relating to the GM plant (III.D) and also the more detailed guidance on comparative and toxicological analysis. Whereas guidance in chapter 7 is quite detailed, it seems to be much less so in chapter 9. The guidance in chapter 7 refers frequently to existing OECD and other guidelines, but in chapter 9 there is only reference to “an example” (SCP, 1999) of how the risk assessment could be done. We hope that in the near future more detailed guidance will be provided on other frequently discussed environmental safety assessment issues (e.g. non-target studies, the interplay between Directives 2001/18/EC and 91/414/EEC, whether and how exposure (by cultivation) to multiple GM plants over the long term should be addressed and guidelines for field trials). “Renewal applications” are not included in this Guidance Document. We would have appreciated that this update provides clarification on the specific requirements of the different renewal type applications (whether there is a prior EFSA opinion of this event, or the event is included in a parallel stacked event application, or there is no prior or running application evaluated by EFSA …). In several sections wording should be more carefully chosen: for instance, in 7.1.1 (line 957-1002) “should include” (957), “would include” (959) and “could include” (992) seem to be used interchangeably, although there is a clear difference in meaning. In line 1074, “is recommended” is used; this is not the same as “is required”. Inappropriate wording may not allow the applicant to understand what is the minimal data he is required to submit (“need to know”) and what is “good to know”. We therefore advise carefully checking the wording of the document. We also advise to check the spelling of the document and the references. A list of acronyms would be useful. We make here some proposals for improvement in terms of content and structure (see specific remarks) and text (see editorial remarks).
V. REFERENCES	Editorial comments Line 3119: include "," after FDA; remove brackets around 2001 Line 3138: include "," after ILSI Line 3179: thesting and add "." after 1998
4. THE RESULT OF RISK CHARACTERISATION	Editorial comments Line 2897: delete "/event(s)" (for reasoning see comment on line 659-666) Line 2909: post-market
3. ISSUES TO BE CONSIDERED FOR RISK CHARACTERISATION	Line 2736: Reconsider if it is not better to replace "risk characterisation" by "risk assessment". Generally, one says that risk assessment is done in a holistic manner and on a case-by-case basis. Line 2739-2740: Here is stated "Below a number of issues are described for consideration in the risk characterisation step". To our opinion, the issues considered are not taken into account in the risk characterisation step, but in the

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	steps before e.g. "molecular characterisation" in hazard identification step, "food/feed safety in relation to intake" in the exposure step. Risk characterisation considers the outcomes of the steps taken before. Hence, we propose to change "risk characterisation" in "risk assessment" in line 2740. Moreover, further down in text, one speaks of risk assessment and not risk characterisation. Line 2743-2746: Characteristics of donor and recipient, and previous use are generally not considered as "molecular characterisation" data. Therefore, we propose to change the title "molecular characterisation" into "information relating to the GM plant". Lines 2750 to 2753 state: "Where flanking sequence analyssis has identified chimeric ORFs, it should be demonstrated how approaches like bioinformatic analysis,...contribute to the safety impact." Firstly, these approaches cannot contribute to safety impact, but rather to safety analysis or to hazard identification. Secondly, what is expected as a demonstration that these approaches contribute to hazard identification? An example would be welcome. Lines 2753 to 2755 state: "The value of the results obtained should be evaluated in the light of the available knowledge on the structure and function of genomic databases of the crop species in question". Firstly, what is meant by "function of genomic databases"? Secondly, why restrict to databases of the crop species in question? For several crops the genomic databases are rather poor, so the use of databases of related species will be informative. Line 2812: The sentence starting on line 2812 “Where the occurrence of unintended effects cannot be excluded…” is somewhat ambiguous. To our opinion, the occurrence of unintended effects in GM derived foods/feed can never be excluded. Editorial comments Line 2776: what is meant with "laci o"? Line 2790: delete "." after "." and before "In addition" Line 2799: analyzsed Line 2804: fertilizsation Line 2820: dose-response Line 2845: replace "produced" by "done"
2. HOW TO CARRY OUT THE RISK CHARACTERISATION	Line 2695: Please replace "risks" by "hazards" confer terminology used in EC, 2000a. Line 2720: Here one uses the term "final risk estimation" instead of "final risk characterisation". Please include the term in the definition of risk characterisation (e.g. in II.4.1.4 risk characterisation/estimation) or omit the term in the text as it might be seen as another step then risk characterisation by people not familiar with risk assessment. We propose to include the term, as it is the term used in the Directive. Line 2730, please reconsider the term "risk estimations"; this could also be replace by "risk characterisations". Line 2703-2704: move "using laboratory...and field trials" to 4.1.2 Editorial comments Line 2702: replace "focused" by "based" Line 2705: comprehensive should not be in italic Line 2706: replace "several approaches including". Molecular analysis,

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	agronomical analyis, etc. are not "approaches". Line 2712: should "iterative" be in italic? Line 2721: should "uncertainties" be in italic?
1. INTRODUCTION	IV.1. Introduction Line 2675-2692 Line 2678: make reference to section II.4.1.4 where more information can be found on the four steps. As mentioned earlier (see comment on line 688-708), we propose to move the definition to section II.4.1.4. As mentioned earlier (see comment on line 498), in section IV the term integrative is not used except in the title. It is preferable to also use the term integrative in the text of this section. Alternatively, the word "integrative" can be removed from the Guidance Document, what is the preferred option to our opinion. Editorial comments Line 2692: Cchapter
IV. INTEGRATIVE RISK CHARACTERISATION OF GM PLANTS REGARDING FOOD/FEED SAFETY AND ENVIRONMENTAL IMPACT	What is the added value/ information brought about by this section? Many of the definitions are redundant …
7.7. Post-market monitoring of GM food/feed	Line 1879: We propose to delete "toxicological" as the pre-marketing testing is broader (also includes allergenicity and nutritional testing). Editorial comments Line 1866: add "-" between Post and Market Line 1866: remove abbreviation (PMM) as it is only used in 7.7 and not further in text (see also lines 1867, 1875, 1879, 1881, 1885 and 1887). Alternatively, one can replace all post-market monitoring by PMM in text. Line 1870: add "-" between side and effects Line 1878: add "-" between side and effects, place "?" after 2003) Line 1881: add "," after therefore Line 1884: add "," after however Line 1884: realizsed Line 1888: Place "." directly after "cases"
7.6. Conclusion of the toxicological/nutritional and allergenicity assessment	Editorial comments Line 1833: foods Line 1853: add "(are)" after "is"
7.5. Anticipated intake/extent of use	Editorial comments Line 1819: e.g. in italic
7.4.2. Nutritional assessment	see comment III.D.7.4.1.

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of GM feed	Editorial comments Line 1757: feeds Line 1759: e.g. in italic Line 1763: e.g. in italic Line 1765: recongnizsed and e.g. in italic and put "," after ILSI Line 1772: assess the impact onf the feed Line 1780: check word "co-products" Line 1788-1789: title of Report in regular instead of italic Line 1798: replace "," by ";" after 2005 Line 1799: replace "," by ";" after 2008
7.4.1. Nutritional assessment of GM food	The amount/type of information in section 7.4.1 should be equivalent to 7.4.2. Section 7.4.1 contains a paragraph on the potential of GM food (lines 1715-1719) and on what nutritional assessment of GM food should consider (line 1720-1726). Similar paragraphs should be included in 7.4.2, or alternatively paragraphs that apply to both GM food and feed, can be moved to the introduction. Editorial comments Line 1722: Ssection and add ";" at the end of sentence Line 1725: Ssection Line 1728: Ssection Line 1734: Ssections Line 1737: Ssection and replace "it" by "this test" Line 1742: e.g. in italic and Ssection Line 1743: put "," after ILSI and replace "," after 2003 by ";"
7.4. Nutritional assessment of GM food/feed	Editorial comments Line 1698: delete "during hazard identification" (unintended effects are not identified in this stage) Line 1702-1708: check letter size Line 1706: unexpected
7.3.2. Assessment of allergenicity of the whole GM plant or crop	Line 1682-1684: The new guidelines insist that the respiratory allergy risk is taken into account. This is a very good point, but this should concern only the applications dealing with cultivation in the E.U., which is not obvious from the text.
7.3.1. Assessment of allergenicity of the newly expressed protein	In lines 1580-1582, it is claimed that the constituents responsible for allergenicity in foods are proteins or protein breakdown products. The majority of food allergens are proteins indeed. However other components of foods may cause allergy (Hegde et al., 2004) or possibly act as haptens (FAO Corporate Document Repository, 2001). Allergy to ingestion of a non-protein secondary metabolite has been described recently (Pramod et al., 2008). Therefore, we wonder whether the statement “that the constituents responsible for allergenicity in foods are proteins or protein breakdown products” should not be adapted. FAO Corporate Document Repository 2001. Evaluation of allergenicity of genetically modified foods. http://www.fao.org/docrep/007/y0820e/y0820e04.htm Hedge VL, Venkatesh YP , 2004. Anaphylaxis to exipient mannitol: evidence for an immonuglobulin E-mediated mechanism. Clin Expl Allergy 34: 1602-1609. Pramod SN, Venkatesh YP, 2008. Allergy to Eggplant (Solanum melongena) caused by a putative secondary metabolite. J Investig Allergol Clin Immunol 18:59-62. Editorial comments

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	Line 1632: why is specific in italic?
7.3. Allergenicity	The section 7.3.2. should, however, insist more on the fact that for allergenicity testing of the whole plant, each GM plant should be considered as a new entity to be evaluated. This point is particularly relevant for the stacked events. Often, the applicant refers to the parent plants to justify the absence of testing in the stack. However, comparison should be done with the natural counterpart and not with the single event. I may happen that the single event is slightly different from the natural and the stack slighly different from the single, but not in a significant way. However, the two slight differences might become significant between the stacked event and the natural. In the new guidelines, this should be more stressed.
7.2.5. Toxicological testing of the whole GM food/feed	Line 1470-1475: Remove "as well as nutritional deficiencies ... 2008)". In "90-day toxicity study in rodents" toxicity testing is discussed, not nutritional assessment. One can mention the nutritional aspects under 7.4.1. Line 1466: Add "for testing of chemicals" at the end of the sentence, as an explanation why these guidelines need to be adapted. Is the best phrase to use in this sentence "should" or "could" be adapted ("can be adapted" is used in line 1500)? Line 1519-1572 Interpretation of relevance of toxicity tests Lines 1551-1569 are a duplication of the information that is present in 1476-1489 and can therefore be omitted. Lines 1570-1572 are out of the scope of this section. Editorial comments Line 1466: remove Guideline 408 Line 1466: Add "." after 1998). Line 1476: "in vitro" and " in vivo" should be in italic Line 1486: foods/feeds Line 1488: feeds Line 1510: include "," between ILSI and 2003; add "." after 2003) Line 1510: Add "," after ILSI and "." after 2003) Line 1530: harmonizsed Line 1548: etcetera should be in italic Line 1554: "in-vitro" and "in-silico" should be "in vitro" and "in silico" and in italic Line 1571: e.g. should be in italic
7.2.3. Testing of new constituents other than proteins	Editorial comments Line 1428: 2001/79/ EC: remove open spaces between / and EC Line 1434: Mmodified
7.2.1. Standardized Guidelines for Toxicity Tests	Editorial comments Line 7.2.1: Standardizsed Gguidelines for Ttoxicity Ttests Line 1357: emphasizsed Line 1378: add "," after required Line 1395: up-to-date (confer line 1350)
7.2. Toxicology	Under the toxicology section 7.2, it seems that a lot of animal testing will be needed; it would be perhaps useful to mention here explicitly that where possible Reduction, Refinement, Replacement will be considered (see also line 1366)? However, in line 1670-1671 it is stated that the development of animal models should be encouraged, therefore, this may be read as "we need more animal testing..." Editorial comments

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	Line 1313: in-vitro should be in vitro and in italic Line 1313: add toxicological before adverse effects Line 1321: (FOSIE, Food and Chem Tox 40 (2002) 2/3) Line 1323: e.g. should be in italic to be consistent with rest of text; check "e.g." throughout text. Line 1339: add "," between proteins and (ii) Line 1342: Ssection; check "S(s)ection" throughout text.
7.1.7. Conclusion of the comparative analysis	Line 1307-1308: This bullet point is not a conclusion of the comparative analysis, but a recommendation to the applicant and therefore it is proposed to be removed. Editorial comments Line 1306: replace "." by ";" Line 1308: replace "." by ";" Line 1309: Wwhether
7.1.6. Effect of processing	Editorial comments Line 1282: e.g. should be in italic to be consistent with rest of text
7.1.4. Comparative analysis of composition	Line 1214-1215: define, for instance in a footnote) what "a nutritionally significant contribution to the diet" is (for instance X% of the DRI/PRI as defined by Y) X could be for instance 15% Y: As long as there are no European recommendations for micronutrients, Y could be defined as the mean value of the DRI/PRI of the European member states as taken up in the EURRECA paper (Hautvast, 2008). Hautvast J: EURRECA: European micronutrients recommendations aligned-Preparing the way. Eur J Nutr 2008; 47(Suppl 1) 1-40. Line 1229: Lines 1228-1229 mention that "identified allergens should be studied". However, natural allergens are not in the list of compounds to be analysed (lines 1205-1208). Therefore, we suggest to add the word "already identified" to make more clear which allergens are referred to.
7.1.3. Selection of material and compounds for analysis	Editorial comments Line 1241: add bullet point at end of sentence
7.1.2. Experimental design and statistical analysis of data from field trials for comparative analysis	Line 1033: Replace "It is important that the choice of sites of the trials represents as fully as possible the range of receiving environments where the crop will be grown." by "It is important that the choice of sites of the trials represents as fully as possible the range of receiving environments where the crop will be grown, reflecting relevant meteorological, soil and agronomic conditions." A typology of the "receiving crop environments" within the EU would be specially useful: would this be available? What are the criteria implicitely evoked by the sentence: climate/soil environments? Are the farming systems included in the so-called "receiving environments"? Later in the guidance document (line 1938) the following wording is found: "…in order to reflect relevant meteorological, soil and agronomic conditions". We suggest to include these words in the continuation of line 1034 as well. Line 1052-1061: is rather complicated; replications should be never lower than 4, and in case of t=5 (the minimum value) then r=5; in all other cases (t>5), r=4 at least. Line 1078: Replace "control" by "comparator" as this is the word used throughout the document.

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	Line 1082-1091: Statistical analysis - Under line 1086 it is written that “data transformation may be necessary to ensure normality”. For data, for example for data of lodging – which do not fulfil normality, it is not possible to transform the data to a normal distribution. In those cases specific statistic tests have to be used - In this paragraph no information is found on the requirements of the validity of the trials (regularity in the field, variation coefficient of the yield,..). Should this not be stipulated in this paragraph? Editorial comments Line 1007: focussing Line 1008: replace genetically modified by GM Line 1038-1039: ''per se'' should be in italic Line 1044: recongnizse Line 1045: maximizse
7.1.1. Choice of the comparator	Lines 957-958: Comparison of the composition of the transgenic plant with a non-GM comparator grown under the same regimes and environmental conditions is obviously very interesting from the scientific point of view. However, for risk evaluation it is probably sufficient to prove that the composition of the GM material is within the range of commercial samples of the same species. (comment also made under II.2.2) Line 992: Here it is said that "The appropriate comparator for the stack could include a non-GM line...'' However, the EFSA Guidance Document on stacked events is more strict on this matter and states ''the most appropriate comparator(s) ... should include ... non-transgenic genotype(s)''. Hence, we propose to change the word ''could'' into ''should''. Line 996-1002: This paragraph deals with risk assessment of stacked events in general and not with comparative analysis. Therefore, we propose to delete this paragraph here and move it to section II, which discusses risk assessment in more general terms. Editorial comments Line 978: Guidance dDocument Line 980: check spacing between ''link'' and ''Where''. in 7.1.1 (line 957-1002) “should include” (957), “would include” (959) and “could include” (992) seem to be used interchangeably, although there is a clear difference in meaning. In line 1074, “is recommended” is used; this is not the same as “is required”. Inappropriate wording may not allow the applicant to understand what is the minimal data he is required to submit (“need to know”) and what is “good to know”. We therefore advise carefully checking the wording of the document.
4. THE RESULT OF RISK CHARACT ERISATION	Under section 4 line 2905 potential differing effects between wild animal fauna and livestock consumption of GM feeds could be separated.
7.1. Comp arative analysis	The section on ''comparative analysis'' is embedded in the section on ''information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed''. However, comparative analysis in particular of agronomic and phenotypic characteristics are also of importance for assessment of the impacts on the environment. As comparative analysis is important for assessment of both the impacts on the environment and on human/animal health, we propose to deal with comparative

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	analysis in a separate section. In this way it is no longer solely dealt with in the section on GM food/feed assessment. Editorial comments Line 955: replace ";" by "."
6. General recommendations	Line 940-941: it is not explained how risk assessment may be simplified: since this is not clear, it may create uncertainty, and this has to be avoided. What is ment by "minimized" in line 941?
5. Conclusions of molecular characterisation (Sections C and D1-4)	Editorial comments Line 928: Ssections C and D1-4 (use either section or Section throughout text)
4. Genetic stability of the insert and phenotypic stability of the GM plant	Editorial comments Line 927: Ssection (use either section or Section throughout text)
3. Information on the expression of the insert	Line 888-890: Information on the parts of the plant where the insert is expressed should ALWAYS be given, and not only when potential risk is identified (as written in the guidelines). Also, it is important that the range of concentration is always given for the EDIBLE parts of the plant but also in pollen when the application concerns cultivation in the E.U. This is important for allergenicity evaluation. Line 893: "…expression of the inserted gene(s) is …stable in the tissue targeted." How should expression stability be demonstrated: how many replicates/generations tested? Should the same materials as used for section III.D.4 be used for these expression studies? Line 894 (c) Potential creation of fusion proteins The creation of fusion proteins is discussed under D.2. Here - in the chapter on expression - one should focus on the potential expression of fusion proteins. Hence, the proposal to change the title of (c) into "Potential expression of fusion proteins with possible safety concern" and the text into "Bioinformatic tools and RNA expression studies may be used for investigating the possible or actual expression of the new ORFs, on a case-by-case basis." It would be preferable if the amount of new/modified protein is always expressed in the same units. Presently, part of the dossiers use fresh weight, others express the amount of proteins on a dry weight base. This situation makes it sometimes difficult or even impossible to compare data between different dossiers dealing with the same organisms and the same modifications. Line 907: Replace "…stacking of events by conventional crossing …" by "…stacking of events by conventional crossing or by other means …" Double transformation or somatic hybridization are other ways of stacking genes that should be taken into account. Editorial comments Line 904: Ssection (use either section or Section throughout text)
2. Information on the	Line 847: Replace "…at the insertion site …" by "…at each of the insertion site …". There can be more than one insertion.

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sequences actually inserted or deleted	Line 849: Replace "…size and function of the deleted region(s)" by "…size and function of the deleted region(s), whenever possible." In some cases, it will be impossible to reconstruct the pre-insertion locus and to accurately identify the deleted region. How to do then? Line 852: Segregation analysis should be used to confirm subcellular localisation of inserts. The relevant genetic information will in numerous cases be available. However, segregation analysis is not always easy to perform (e.g. plants with a long life cycle) or even impossible (non-fertile plants). In these cases, the analysis of the sequences flanking the transgenes should be sufficient to confirm the subcellular localisation. Line 860: Add : When identifying the new ORFs, no minimal size criterium should be used but all ORFs should be considered instead. An ORF may defined as any nucleotide sequence that contains a string of codons that is uninterrupted by the presence of a stop codon in the same reading frame." The definition of the ORF could be given as a footnote. The recurrent question of a minimal size for the ORFs to be considered when searching for homology with known toxins or allergens should be clarified in the proposed way (or some other way). Line 860: Add "any ORFs newly created … fusion proteins, or due to internal insert rearrangements or to tandem repeats of the insert within genomic DNA." New ORFs may be created in other locations than at the junctions with receiving plant DNA. Line 862: "…using up-to-date databases…" The origin and versions of the databases should be explicated in the dossiers. Editorial comments Line 857: abbrevition of ORF can be put in text as has been done for other abbreviations
7.7. Post-market monitoring of GM food/feed	Under section 11.4.3. it should be emphasized that a baseline is required for both the GM organism and the environment receiving the organism.
3. Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion	Editorial comments Line 819-820: lettertype should be adjusted (cfr e.g. 7.1) Line 828: lettertype should be adjusted
1. Description of the methods used for the genetic modification	III.C.1: Include (e) the helper plasmid if used during the genetic transformation process. This type of information was required in the EFSA Guidance Document, 2006.
B. INFORMATION RELATING TO THE	Line 761: "the most recent taxonomic classification should be used": We suggest "the most recent accepted classification" Lines 774-775: "information on natural predators, parasites, competitors and

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RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS	symbionts": this is unclear. What is meant with "of the plant"? What is a "predator of a plant"? With "parasites" is meant "pests of the plant" or "parasites of the plant's pests"? Editorial comments Line 785: e.g. should be in italic
A. GENERAL INFORMATION	Editorial comments Line 723: III B should be IIIB (is sometimes put together, sometimes put apart in text) Line 726: Regulation (EC) No 1829/2003 Line 726: delete one "to" Line 729: Regulation (EC) No 1829/2003 Line 730: Regulation (EC) No 1829/2003 Line 733: Regulation ..., Article (write full-out)
4.2 Issues to be considered for the Risk Assessment of GM Plants	Editorial comments Line 709: Rrisk Aassessment of GM Pplants
4.1.4. Risk characterisation	Line 689-708: While in 4.1.1, 4.1.2 and 4.1.3 the step is defined, a definition is missing for risk characterisation. We propose to include the definition of available on lines 2679-2682 here. To avoid overlaps with section IV, we propose to remove lines 693-697 and 698-704. As for 4.1.1, 4.1.2 and 4.1.3, one could keep the information restricted to one single paragraph. Reference can be made to section IV for more information. Line 696: Why are nutritional aspects mentioned separately? This aspect falls under "effects on humans/animals" and it is not necesarry to mention this separately. Line 705: the word "uncertainties" could be included: "The risk characterisation should also include uncertainties and indicate when a..."
4.1.3 Exposure assessment	Line 677-687: This paragraph is mainly focused on exposure assessment of GM food/feed and should be broadened to environmental exposure assessment. We propose to change line 678 as follows: "...exposure to humans, animals and other organisms to the GM plant and/or derived products...". In addition, some lines on exposure assessment of organisms other than humans/animals could be added. We propose to delete sentences line 681 to 686 "For exposure assessment... quality.", as this information is redundant here and we propose to refer to D 7.5 for more information. In line 687 reference should be made to D.7.7 for post-market monitoring issues. Editorial comments Line 679: include animals: "With regard to humans/animals..."
4.1.2 Hazard characterisation	Line 668-675: Line 671: replace "toxicological/nutritional potential" by "adverse effect potential", as the former phrase is too restricted and only covers potential adverse effects of GM food/feed and not of the environment. Line 674: delete "or nutritional". We don"t see the need to stress nutritional adverse effects; these are covered under the word "adverse". Editorial comments Line 668: characterizsation Line 668: Delete "The" and "step", resulting in Hazard characterisation is defined as... Line 668-670: why is part of sentence italic?

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	Line 670: add brackets around "a" and at the end of sources ... (a) risk source(s) Line 671: focussed. Either use focussed or focused in text, but not both, check throughout text. Line 675: dose-response
4.1.1. Hazard identification	Line 659-666: We propose to leave out "events" in lines 660 and 661. What is meant with this? This word is also not commonly used in the rest of the Guidance Document (also delete the word in line 2897). The following change should be made (line 661): "... together with a qualitative description of the risk sources". In the hazard identification step, the characteristics that might cause an adverse effects are described, not the adverse effects. Editorial comments Line 659: add brackets around "a" ... identification of (a) risk source(s) Line 661: add "," after EC ... (EC, 2000a) Line 662: focussed Line 663: comparator(s)
7.7. Post-market monitoring of GM food/feed	Under point 9 it could be added two new headlines "Dispersal" and "potential effects on ecosystem functions".
4. THE OBJECTIVES OF THE DIFFERENT STEPS OF THE RISK ASSESSMENT PROCEDURE FOR GM PLANTS AND DERIVED FOOD/FEED AND ISSUES TO BE CONSIDERED	see comment in section II.3
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Section II.3 and II.4 need revision for the following reasons: - One might have the impression that II.3 deals with risk assessment of the environment and II.4 with risk assessment of GM food and feed, when looking at the titles of both chapters. However, also environmental safety assessment is dealt with in II.4. This possible confusion can be dealt with by changing the titles of section II.3 and II.4 (e.g. II.3 in "risk assessment and monitoring" and II.4 in "The objectives of the different steps of the risk assessment procedure for GM plants"). - The content of section II.3 shows overlap with the information present in section II.2 (for lines 617-619) and section II.4 (for lines 623-641). Moreover, lines 623-641 describe an example of how to conduct risk assessment for (non)-target organisms. In section II, risk assessment should be described in general terms covering all points mentioned in section III.D.9. To our opinion, lines 617 to 641 can be omitted and parts of it transferred to II.4.1. - The focus of II.3 could be on monitoring and its relationship with risk assessment; the focus of II.4 on risk assessment: its steps, principles (to be included) and issues to be considered. If this approach is taken, it is preferable to first discuss risk assessment (II.4) and then monitoring (II.3)

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	Editorial comments Line 626: replace "and" by "or": "GM plant or its products", as organisms are not necessarily exposed to both. Line 640: add "," between while and Ttier Line 640: If a hazard is an “agent having the potential to cause an adverse effect” (see footnote under Line 309), what is a potential hazard ?
2.3 Intended and unintended effects	Editorial comments Line 595: single is in italic: why? Line 596: targeted is in italic: why?
2.2 Concept of substantial equivalence or comparative safety assessment	Lines 570: The second part of the safety assessment procedure should not only include further specific toxicological and nutritional testing, but also allergenicity testing. Taking into account that this aspect is thoroughly addressed in the Guidance Document, it is suggested to add allergenicity testing in the present sentences. Lines 573-574: Comparison of the composition of the transgenic plant with a non-GM comparator grown under the same regimes and environmental conditions is obviously very interesting from the scientific point of view. However, for risk evaluation it is probably sufficient to prove that the composition of the GM material is within the range of commercial samples of the same species (comment is repeated under III.D.7.1.1). Editorial comments Line 569: comparator(s)
2.1 Concept of familiarity	In point 2.1 and 2.2: the use of a non-GM crop as comparator is mentioned, but the variation between cultivars, types, variants of the same plant may be much bigger than differences between the GM an non-GM comparators. This is not addressed here, but this is discussed under 7.1. Therefore, we propose to add reference here to 7.1.
2. COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF GM PLANTS	Line 547: Include "phenotypic characteristics". One also speaks of "agronomic and phenotypic characteristics" in section 7.1. Line 559-560: Non-GM lines may not be the only comparators. This is true for stacked GM plants for example, where single events are useful. It is mentioned that “similar constructs and traits in similar or different crops may be used for assessing familiarity, hence GM comparators should be admitted for coherency: “…with their non-GM comparators and with GM comparators when appropriate.” Editorial comments Line 538: change lettertype of GM
1. INTRODUCTION	Line 488: It is preferable not to speak of " identification of risks", but only of "identification of hazards". The latter is the term used in the document (see 4.1.1) and Directive 2001/18/EC, and is the best term to be used as explained in lines 513-518. The sentence (line 488-491) could be simplified as: "Risk analysis (risk governance) follows a structured approach and consists of three basic elements: ...." Line 493-501: In this paragraph risk assessment is described/defined. The definition mentioned here is not the one that is present in Commission Decision 2002/623/EC, hence we propose not to make reference to EC, 2000a in line 496. We cannot agree with the wording "the identification of the attendant uncertainties

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	(line 494)": not uncertainties are identified, but hazards and potential adverse effects. We propose the following changes: "Risk assessment can be described as a process of evaluation of risks to human and animal health and/or the environment (in the current definition it is not clearly mentioned what is evaluated) and comprises of four steps: hazard identification, ... (Please refer to 4.1 for the definitions of the four steps)". As mentioned earlier, risk assessment (and also risk management and risk communication) have been defined in footnote 4. Line 498: What is meant with integrative risk characterisation? This term is also not further explained/used in II.1, where one speaks of final risk characterisation (line 530) and not integrative risk characterisation. To make this new term more clear, we propose to also include the term "integrative" in line 530 (reading thus as "The final integrative risk characterisation...") and to make reference to section IV on integrative risk characterisation. However, also in section IV, the term integrative is not used, except in the title. It is preferable to also use the term integrative in the text of this section. Alternatively, the word "integrative" can be removed from the Guidance Document, what is the preferred option to our opinion. Editorial comments Line 490: add "," after EC and replace "," by ";" after 2000a Line 490-491: why is part of sentence in italic? Line 491: add "," after "Alimentarius" Line 496: add "," after (EC Line 498: replace "," by ";" after 2000a Line 500: Cchapter Line 525: Fosie, 2003 is not mentioned in reference list Line 528: non-GMO"s should be non-GMOs Line 529-530: remove "In order to carry out the risk assessment". By doing this one avoides two times "in order to" in one sentence.
II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS	To improve consistency in the titles of the sections and subsections, we propose: - to change the title of section II into "PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GM PLANTS AND/OR DERIVED FOOD AND FEED". In the introduction is it is clarified that this guidance document is a document on GM plants. Hence, we think it is better to reflect this in the titles of the document, and not to use GMO in the title of section II. The title we propose is more in line with the title of section III. The title of this section is "Principles and strategies for risk assessment of GMOs". However, in this section no specific information is given on the general principles on which the risk assessment should be based. Some of the principles of risk assessment are addressed in I.2. Deliberate release of GMOs. One can state in this section that the risk assessment of GM plants and derived food and feed (see also Annex II on the ERA and its the guidance note (2002/623/EC) of Directive 2001/18/EC and Gray (2004), should: - be science-based, thus carried out in a scientifically sound and transparent manner based on available scientific and technical data, - respect the precautionary approach meaning that if there is any doubt one attempts to resolve it, and if there are irresolvable uncertainties one attempts to make them explicit, - use a step-by-step approach ensuring a gradually increasing familiarity with each GM crop as it moves from containment to the greenhouse and experimental

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	release to commercialisation, - define a baseline to allow comparison, - be carried out on a case-by-case basis dealing with each crop-construct combination separately, - be iterative and continuous, capable of responding to new information. We are of the opinion that it is better to address and explain these general principles in section II of the Guidance Document. Gray A (2004) Ecology and government policies: the GM crop debate. Journal of Applied Ecology, 41: 1-10
4.2 Issues to be considered for the Risk Assessment of GM Plants	It could be added "the possibilites for monitoring potential effects on the environment". Moreover, in line 713 it could be added .../and its potential range/.../.
2. LEGAL BACKGROUND FOR THE RISK ASSESSMENT OF GMOS, GM FOOD AND GM FEED AT COMMUNITY LEVEL	Line 309 Footnote 4: Here hazard, risk, risk analyis, risk assessment, risk management and risk communication are defined. We want to note that risk analysis, risk assessment, risk management and risk communication are again defined further in text (see II.1). We propose to keep solely one definition. Comparison of both texts shows differences: e.g. about risk management, “in consultation with interested parties” is mentioned in the footnote, but not in the II.1 section. Line 387-404: Please replace "application" by notification and applicant by "notifier" as these are the terms used under the Directive. Remove line 392-393 "The principles for the ... of the Directive". Principles are already dealt with in the first paragraph (line 372-380). One can mention Annex II in line 373. Editorial comments Line 300: add "No" after (EC) Line 313: add reference EC, 2003a after Regulation (EC) No 1829/2003 Line 319: add reference to Regulation (EC) No 258/97 (also in reference list!) Line 323: add reference of Directive 90/220 and 2001/18 Line 339: "Regulation for GM" should be "Regulation of GM" Line 349: add reference to Regulation (EC) No 178/2002 Line 353: leave out (No); add EC after 2001/18 Line 359: EFSA"s Line 373: leave out "of the European Parliament and of the Council". This is not mentioned for other legislations Line 382: leave out e.g. Part B = field trials Line 390: III B should be IIIB (is sometimes put together, sometimes put apart in text) Line 441: add "No" after (EC) Line 462: add "No" after (EC) Line 469: add "No" after (EC)
1. SCOPE OF THE DOCUMENT	Line 255-256: These lines quote: "When a product is likely to be used both for food and feed purposes, the application should fulfil the authorisation criteria for both food and feed". As formulated here, the possibility remains that a GM plant intended to be used for feed purposes will only be evaluated for feed safety aspects and not at the same time for food safety aspects. Since the sentence can be read as "When a product

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	is used for feed purposes, the application should fulfil the authorisation criteria for feed". According to the GM food and feed regulation, authorisation should not be granted for a single use when a product is likely to be used both for food and feed purposes; therefore such products should only be authorised when fulfilling authorisation criteria for both food and feed. The present sentence should be rephrased considering the provisions of the Regulation (EC) No 1829/2003 on GM food and feed. Editorial comments Line 254: place (EC, 2003a) after feed. Line 257: III B should be IIIB (is sometimes put together, sometimes put apart in text, check throughout text) Line 268: Uupdated Line 268-270: delete "of the GMO Panel on the risk assessment of GM plants and/or derived food and feed including its updates" and "n updated". Include "of the GMO Panel" after Guidance document. This will result in the following change: "This updated Guidance Document will be a replacement of the Guidance Document of the GMO Panel for the risk assessment of GM plants and derived food and feed". Line 271: reference should be EFSA, 2006c Line 272: This guidance document provides detailed guidance... Line 280-282: Add Directives and/or Regulations to which is referred as done in paragraph 286-292; or leave them out in the latter paragraph.
TERMS OF REFERENCE	Editorial comments Line 231: replace genetically modified by GM Line 231: add reference (EC, 2003a) after feed Line 232: remove "the European Food Safety Authority" and retain only abbreviation.
1. SCOPE OF THE DOCUMENT	The applicant frequently claim Confidential Business Information (CBI) on extensive parts of the data supporting the safety of their product. Biosafety data should in general not be labelled confidential as this limits transparency, public trust in the process, and prevents independent peer review and assessment of the methods used. Thus, it greatly reduces the scientific quality of the submitted data as independent and open peer review is an integral part of the sound scientific process. To my opinion, there is a great variation of the extent CBI claims are made on biosafety data, and what is accepted by EFSA is uncertain and likely y a variable of various unknown factors with little transparency in the process. In the interest of ensuring the highet safety level of products entering the European market, the Draft opinion should contain a section on how EFSA deals with CBI claims, and each following section of the document should have a separate paragraph attached outlining what type of information that can be kept confidential. For instance, data submitted by the applicant related to human exposure, toxicology, allergenicity environmental safety and monitoring should not be allowed to have CBI claims attached. Finally, many developing countries with substantially less resources and regulatory

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	capacity will use the EU document for guidance, and a clear definition of the scope and limitation of CBI claims will be very useful for their in-country assessment procedures too.
OTHER SCIENTIFIC COMMENTS	III D.12: ERA of plants containing transformation events combined by conventional breeding. We retain our view (expressed in response to a previous EFSA consultation on the risk assessment of GM plants containing stacked events) that there is no scientific justification for the position that EFSA has taken on the environmental risk assessment of plants containing stacked GM events. We appreciate that the amount of data (which is additional to that already provided in notifications for individual GM events) may differ depending on the relationship between the stacked genes and their products. For example, GM crops transformed with genes from the same biochemical pathway may well require further analysis as the GM phenotype may not be the sum of the two parents (within the range of biological variation). However, it is likely that these 'parental GM lines' will not be commercial lines in themselves and that their phenotypes will be assessed as segregants in the application for the stacked product. There is no scientific justification for requesting additional data in the ERA for GM crops in which the stacked transgenes encode products that are not affected by their mutual presence e.g. genes encoding Bt and HT traits. EFSA's line that more data are required (other than that needed to confirm the molecular identity of the stacked events) in every case, is based on speculation and not on scientific evidence. In notifications for GM plants containing stacked events, applicants should: (1) discuss whether there is any interaction between the products encoded by the transgenes. Where there is no scientific basis for this being the case, then there is no a priori reason for providing additional data in the ERA. (2) discuss the potential for the combination of GM traits to impact on the environment. This, for the most part, should be based on data and information provided in notifications for the individual events e.g. the potential for altered management practices to be used in association with stacked HT traits and the consequent environmental impact. It is conceivable that more data would be required if a GM trait affected the previous ERA of another trait in the same plant. For example, a GM plant expressing an insecticidal protein may be cultivated in a different environment from that considered previously if it was then crossed with a GM line containing a gene conferring tolerance to an abiotic stress.
OTHER SCIENTIFIC COMMENTS	Section III. 11. Environmental monitoring plan We agree with the principles for the post-market monitoring of GM crop releases described by EFSA. However, this section could be condensed. It is overly long and repetitive, which gives it disproportionate weight compared to sections on ERA. III D.11.2 Line 2259. We consider that the main objective of case-specific monitoring is to test assumptions regarding occurrence and impact of potential adverse effects of the GMO or its use in the environment. This is consistent with a later statement in the guidance (line 2293) that explains that case-specific monitoring is not indicated if there is a negligible degree of uncertainty in the ERA. III D.11.4 line 2312, the guidance refers to the definition of significant environmental damage used in the Environmental Liability Directive. We note that

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	the GMO Panel adopted this criteria in its opinion on Hungary's safeguard action on MON810 maize. We welcome this pragmatic approach and would support a more categorical recommendation in the guidance, which should be discussed by Competent Authorities under Directive 2001/18/EC. III D. II.5 line 2585. This is inaccurate. Directive 2001/18/EC does not specify what information farmer/growers are required to provide in national cultivation registers.
OTHER SCIENTIFIC COMMENTS	Section III. 9.9 Impacts of the specific cultivation, management and harvesting techniques We are content with this section but are aware that the Panel has drafted a working document on the interplay between Directives 2001/18/EC and 91/414/EEC, which is relevant. xxx has encouraged the Commission to circulate this document to Member States as soon as possible.
OTHER SCIENTIFIC COMMENTS	Section III. D 9.8: Effects on biogeochemical processes Whilst lines 2099-2104 and 2109–2111 in Section III D. 9.8 are fine (with the emphasis on 'where appropriate'), the examples (starting at line 2104) are misleading. Biological nitrogen fixation is not of great importance in cultivated agricultural systems unless they include legumes. This is because N fertilizers provide more nitrogen. The N cycle is important and nitrification and denitrification are key processes that regulate inter-conversion by nitrification of ammonia (less mobile/less available to plants) to nitrate (more mobile in soil and likely to be lost by leaching). Furthermore, nitrate is lost due to denitrification which results in gaseous oxides of nitrogen (potent greenhouse gasses) and nitrogen gas. These are not rhizosphere processes and are influenced by soil properties, particularly the available nitrogen and soil moisture. It is crop and soil management (which may be different for novel GM crops) that will influence these processes more than the crops themselves.
OTHER SCIENTIFIC COMMENTS	Section III. D 9.1 – 9.7 These are suitably non-prescriptive and fit for purpose. As discussed above, applicants should be encouraged to make use of published literature effectively. This is lacking in many applications and it is not addressed in the guidance.
7.1. Comparative analysis	Section 7.1 Comparative analysis The guidance on statistical design for comparative assessment (7.1.2.c, line 1070 onwards) is a good idea. However, it may not be applicable universally. We support a greater emphasis on a quantitative approach aided by clear guidance on how this can be achieved. However, we consider that there needs to be a degree of flexibility for applicants that allows for the submission of evidence of equivalent power generated by other methods. A decision has to be made (and argued) on where the upper and lower equivalence limits are set as well as on the level of power (as was the case in designing the approach taken in the UK's farm-scale evaluation trials). This will be contentious and as such, we welcome and encourage further discussions on how to set realistic limits. In lines 1026 – 1030, the Panel indicates a need for applicants to investigate the interaction between inherent differences in the GM plant and environmental conditions. However, it does not provide guidance on how these analyses should be carried out. The Panel should clarify whether it is recommending that this interaction is tested for significance, and if so, how it would suggest this is carried out in the context of the analyses presented in the figure described in line 1128.

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4. Genetic stability of the insert and phenotypic stability of the GM plant	We do not consider that there is justification for suggesting that data from five generations are 'normally' required to demonstrate genotypic/phenotypic stability in lines 907-909. This level of prescription is not helpful as it appears to exclude other evidence such as experience of cultivating a GM crop outside of the EU prior to its notification for authorisation in the EU.
3. Information on the expression of the insert	The Panel should explain what it means in lines 893 and 894 and provide an example.
3. ENVIRONMENTAL RISK ASSESSMENT AND MONITORING	Section III. The document is not easy to navigate. A flow chart identifying the sections and the key issues contained within them (including cross-references to the appropriate text) would be useful. The Panel needs to be clear on the difference between seemingly mandatory requirements (e.g. studies on 5 generations to demonstrate genotypic/phenotypic stability) and recommendations. For example, is there any flexibility in the case of the former? Would data from 4 generations be acceptable if the applicant made a strong enough case? If so, how does this compare with the latter? In general, the guidance appears more comprehensive on issues associated with GM food and feed safety than on issues associated with environmental risk assessment (ERA). We appreciate that EFSA has a mandate to consider certain aspects of the ERA in more detail (in particular, potential effects on non-target organisms and development of criteria for field trials to assess the potential ecological effects of the GM plants in receiving environments). However, it is important that the guidance is not prescriptive and that it establishes principles, requiring applicants to justify their approach. For example, in rare cases where third tier tests on non-target organisms of known and sufficient power are available, first and second tier tests may not be required (although this assumption would need to be supported by argument). We consider that applicants should be encouraged to make use of published literature effectively. This is a weakness of many applications and the guidance should address this. It would be useful if applicants were encouraged to provide detailed summaries of the different sources and layers of evidence that support conclusions made under different section headings.
II. PRINCIPLES AND STRATEGIES FOR RISK ASSESSMENT OF GENETICALLY MODIFIED ORGANISMS	As regulators under Directive 2001/18/EC, xxx is concerned that the principles for risk assessment are not necessarily communicated in a manner that is compatible with Directive 2001/18/EC. In particular, risk management is defined as a 'process of weighing policy alternatives..' Council Decision 2002/623/EC, which provides guidance on Annex II of Directive 2001/18/EC explains how risk management is an integral part of the scientific assessment process. A flow diagram would be useful linking the terms referred to in sections II.1, II.4 and IV. Section II.4 describes the different steps in the 'safety assessment' but does not include assessment of risk management/ mitigation strategies, which is one of the steps described in the analysis of environmental risk assessment (Council decision 2002/623/EC). For example, in considering the results of the UK's farm-scale evaluations, we recognised the potential for off-setting impacts through the adoption of 'mitigation' practices. Similarly, the use of refugia is considered to reduce the risk of insect resistance to Bt toxins developing. These measures have a technical/ scientific basis which need to be considered by risk assessors when advising on the overall risk associated with GM crops released in accordance with differing risk management scenarios.