ANNEX I-4

COMMENTS BY THE EUROPEAN COMMUNITIES ON THE REPLIES BY THE SCIENTIFIC EXPERTS TO THE QUESTIONS POSED BY THE PANEL 28 JANUARY 2005

TABLE OF CONTENTS

TABLE OF ABBREVIATIONS

ACRE	UK Government's Advisory Committee on Releases to the Environment
ACT	Australian Capital Territory
AIA procedure	Advance Informed Agreement procedure
ASEAN	Association of South East Asian Nations
BINAS	Biosafety Information Network and Advisory Service
Bt	Bacillus thuringiensis
CA	Competent authority
CTFBT	Codex Alimentarius Commission established an Ad Hoc Intergovernmental Task Force on Foods derived from Biotechnology
Directive 2001/18	Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC
Directive 90/220	Council Directive 90/220/EEC of 23 April 1990 on the deliberate release of genetically modified organisms
DNA	Deoxyribonucleic Acid
ECJ	European Court of Justice
EFSA	European Food Safety Authority
EPA	United States Environmental Protection Agency
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FSE	Crops and Farm Scale Evaluations
GATT 1994	General Agreement on Tariffs and Trade 1994
GEF	Global Environment Facility
GILSP	Good industrial large-scale practice
GM foods	Food products containing, consisting or produced from GMOs
GM products	Genetically modified products
GMHT	Genetically Modified Herbicide Tolerant
GMOs	Genetically modified organisms
HT	Herbicide Tolerant
IANB	UN Inter-Agency Network for Safety in Biotechnology
ICPM	Interim Commission on Phytosanitary Measures
IOE	The International Office of Epizootics
IPPC	International Plant Protection Convention

LMO-FFPs	Living modified organism intended for direct use as food or feed, or for processing
LMOs	Living modified organisms
NAS	United States National Academy of Science
OECD	Organisation for Economic Cooperation and Development
OSR	Oilseed rape
Regulation 258/97	Regulation (EC) N° 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
SCP	Scientific Committee on Plants
SPS Agreement	Agreement on the Application of Sanitary and Phytosanitary Measures
TBT Agreement	Agreement on Technical Barriers to Trade
UNEP	United Nations Environmental Programme
UNIDO	The United Nations Industrial Development Organisation
WHO	World Health Organisation
ORF	Open Reading Frame (Protein Coding Sequence of the DNA)

I. INTRODUCTION

1. In this submission the European Communities responds to the invitation by the Panel to comment on the advice provided by the Panel's experts, in the form of comments on their replies, and, where appropriate, in the form of further scientific and technical evidence in relation to the Panel's questions.

2. The European Communities has taken very seriously its task of providing science based comments, to contribute to the Panel's understanding of the expert replies. In this regard the European Communities has sought to rely on the most up-to-date science available. For the purpose of ensuring scientific and technical accuracy of its comments, the European Communities has also relied upon independent scientific advice from internationally renowned scientists, as appropriate.

3. By way of introduction, the European Communities is pleased to note that the replies provided by the Panel's experts very largely confirm the Communities' view that the scientific and technical issues involved in considering whether to authorise the release into the environment and the marketing of GM products are very complex and often controversial. The independent experts have confirmed that the science has developed considerably over the last ten years and is still evolving at a fast pace. And most significantly they have confirmed that each case is to be taken on its own merits, and indeed that each case was taken on its own merits. This in turn confirms the approach taken by the European Communities and the Member States and is inconsistent with any claim as to a 'moratorium'.

4. The European Communities does not however agree with all the advice and every answer (or specific parts thereof) provided by the experts, and will comment in detail below. These comments follow the order of the Panel's questions and are divided into sections corresponding to the main issues as identified by the Panel (Sections IV to VIII below).

5. At this stage, the European Communities will provide comments only in relation to a number of replies, as, on these, it considers appropriate to submit them in advance of the meeting with experts. This approach is intended to contribute to the Panel's understanding of the experts' replies, having regard also to the time resources available to comment within the rather tight deadlines.

6. One of the consequences of the lack of time is that this submission is not as concise and well prepared as the European Communities would have wished. Some repetition has been unavoidable and although this submission is long, it is not complete. The European Communities reserves its right to revisit its replies in due course, as well as other replies and questions, and provide further comments and scientific or technical evidence, as provided for in paragraphs 13 and 14 of the Panel's *additional working procedures for consultations with scientific and/or technical experts*. The absence of any response or comment in respect of an answer to any question should not necessarily be taken as acceptance on the part of the Communities of the adequacy or accuracy of the response to the question.

7. Before commenting on the replies to the questions, however, the European Communities comments on the varying approaches taken by the various experts in undertaking their task and on some general and methodological issues (Sections II and III). As will be explained below, this has a bearing on the interpretation of the advice, on the weight to be given to the various expert opinions, and consequently on the Panel's ability to make findings of fact on that basis.

8. In this submission, the European Communities does not discuss the questions of definitions on which international organisations have recently provided advice. As requested by the Panel in its letter of 22 December 2004, these issues will be the subject of comment in a separate submission that the European Communities will present to the Panel on 4 February.

9. The European Communities welcomes the opportunity to discuss these scientific and technical issues further with the Panel and the experts on 17 and 18 February. On this occasion it reserves its right to provide the Panel with further scientific and technical evidence in response to the many aspects of the questions that remain unanswered. The European Communities may bring some additional scientific and technical experts to that meeting as part of its delegation.

10. The European Communities is providing only one exhibit to this submission, Exhibit EC-156, containing the unpublished scientific literature referred to herein. Other documents that are publicly available are not exhibited.

II. THE ADVICE AVAILABLE AND THE VARYING APPROACHES OF THE EXPERTS TO THEIR TASK

11. The European Communities wishes, through the Panel, to thank the Panel's experts for their valuable contributions, which was extremely difficult in the light of the amount of information to process, the number of issues to address, as well as the number of scientific and technical disciplines to cover. The Communities is especially conscious of the fact that responses were provided within the very short timeframe provided for in DSU proceedings.

A. THE INCOMPLETENESS OF THE RESPONSES

12. It is therefore not surprising that the Panel's experts could not cover all necessary disciplines and aspects of the Panel's questions, and provided in several instances answers only on certain aspects (or occasionally no answer at all), in accordance with what they had already made clear in their correspondences from last November. They provided replies, sometimes with detailed and extensive elements on some specific aspects, which, all together, left a large number of elements of the Panel's questions unanswered.

13. There are at least 18 questions which are either not answered at all (e.g. 2, 26, 114), or for which necessary elements of the replies are explicitly omitted by the experts. There are many more, as identified below in the detailed comments of the European Communities, where necessary elements of the replies are implicitly omitted by the experts, in accordance with their own scientific or technical expertise, and/or their initial commitment identified in their correspondence of last November.

14. The European Communities has endeavoured to collect the necessary scientific and technical evidence to provide the Panel with complementary elements of replies to the many aspects that remain unanswered. It has not, however, had the necessary time to submit all this information at this stage, and will seek to provide further evidence at the meeting with the Panel and the experts, if appropriate.

15. The European Communities also regrets, in light of the complexity of the advice sought by the Panel, and more generally of this case, that often only one opinion (and sometimes a partial one) has been made available to the Panel on many questions, or parts thereof. For many questions a number of scientific disciplines and issues are involved, and these do not allow for much overlap between the limited number of selected experts. It is doubtful whether the Panel can make definitive findings of fact on the basis of advice which is, on its own terms, so limited and incomplete.

B. THE DIFFERENT APPROACHES OF THE EXPERTS

16. The Communities notes that the Panel's experts have taken very different approaches, and frequently disagree among themselves, as indicated below. This confirms that there is legitimate room for disagreement on many of the issues. Such disagreement contributes to uncertainty and is bound to be taken into account by the Panel as it carries out its tasks.

17. After consultation and peer review with several independent scientists which all concur with the same opinion, the European Communities has come to the firm view that, among the four experts which were allocated the whole set of questions, only three (Dr. Andow, Snow and Squire) have adopted a balanced and fully reasoned approach, including an indication of their processes of thinking. These experts have provided for transparency on their conclusions and well-structured replies. They have critically evaluated the available information, questioned claims, and presented their own rigorous and logic scientific thinking, based on their own expertise, and each within the limited framework of their respective scientific disciplines.

18. Dr. Andow has been very thorough and plainly has carried out extensive work in preparing his detailed replies, which are often accurate and relevant. He may however on some aspects, as explained below, have overstepped his scientific expert mandate. Dr. Snow and Squire have covered fewer issues, and often in less depth than Dr. Andow, but they have nevertheless also given quite a number of accurate, sensible and fair replies.

19. In contrast, the responses of Dr. Nutti are much less scientific, and largely superficial in character. For the most part, no detailed explanation has been given into the basis for her thinking. Often Dr. Nutti has taken the information provided at face value without personal critical scientific assessment regarding its data basis and validity. As a consequence, her replies, which miss references of the most appropriate work, including as regards Codex references, seem unbalanced and incomplete as they often reach an identical conclusion (usually a systematic pattern of disagreement with any request for more information from Member States). The European Communities is bound to conclude that her replies are frequently inconsistent and often lack proper scientific argumentation.

20. One aspect of her replies is also the low level of information requirements for food safety assessments. Dr. Nutti advocates these in general terms, without proper consideration of the context of the complete information available for each product at that time, and in particular the possible unintended effects that can not be excluded in the face of difficulties, such as missing data, missing assessment or biased analysis, related to the molecular characterization of the specific GM product being considered. As a consequence, her approach addresses only minor parts of possible toxicological, nutritional or other potential food or feed safety problems, and is therefore of limited relevance. Coupled with Dr. Nutti's stated absence of expertise on environmental impacts the European Communities has concluded that the views of this expert should be subject to careful and critical scrutiny.

21. The European Communities judges that the responses by Dr. Andow, Dr. Snow and Dr. Squire provide a more scientific and valuable source of expertise, addressing the issues that justify comment and attention, and that the Panel should most valuably use and take into account to make its findings.

22. As regards the two other experts, who have addressed a limited number of questions, they have provided a reasonable insight into their own scientific thinking. The European Communities is struck by the number of disagreements identified in their respective replies, even in relation to the small number of issues they have addressed. It is noteworthy that they have very different and

opposite requirement in terms of the information necessary to properly assess the molecular characterization of the products in dispute: Dr. Healy seemingly has a much lower threshold of requirement than Dr. Snape. Furthermore, according to the assessment of scientists which are specialized in and working on detection methods, Dr. Healy is often inaccurate and has almost a systematic pattern of advice against the European Communities in her few replies addressing this issue of detection methods.

C. THE INDEPENDENCE OF THE EXPERTS

23. The European Communities would like here to note that both Dr. Nutti and Dr. Healy are employed by Government agencies working for regulatory authorities, including one of a third Party in these disputes on the side of the complainants. By contrast, the other scientific experts are independent. Dr. Nutti and to a lesser extend Dr. Healy (for instance on Q25), are also the only two experts who have touched upon issues in a more general way, going beyond their mandate or their own field of scientific expertise.

D. THE ASSESSMENT OF THE REASONABLENESS OF TIME TAKEN

24. Dr. Andow is providing advice outside his field of expertise when he opines on the length of time that would be appropriately taken to formulate an opinion or a request for further information. It is obvious from his own advice that he has demonstrated his ability to process in a very short time and in detail, a very large amount of relevant information. However, it cannot be assumed that such efficiency can be a benchmark for the facts involved in this dispute, which involved complex regulatory frameworks and interaction between multiple authorities within different jurisdictions, or where a single relevant regulatory authority or legislator had to understand complex scientific information on all issues at stake, call for its own scientific independent advice, including through multiple external advisory bodies, process its or their opinions, interact with an applicant, and finally formulate a request for further scientific information.

III. GENERAL AND METHODOLOGICAL ISSUES

25. There are a number of general and methodological issues that the European Communities finds more convenient to deal with separately before embarking on a detailed commentary on the responses submitted by the Panel's experts to each of the Panel's questions. These are dealt with in this Section. Some can be dealt with briefly, but one, relating to the evolution of risk assessment techniques (sub-section P below), needs to be discussed in some detail.

A. COMPLEXITY

26. It is clear from the experts' replies that the science behind any assessment of the products in dispute is complex, and how many different scientific fields of expertise must be involved to address the issues to give a complete picture. In particular, the potential impacts of the products in dispute may apparently only be uncovered and assessed by the scientific analysis of complex networks of food, agro-environmental or ecological webs, involving direct and indirect interactions between many living organisms and systems. This has obviously raised significant methodological challenges for the scientific community, with all that implies for governmental policy and decision-making.

B. CASE BY CASE ASSESSMENT

27. There is overwhelming evidence from the experts' scientific advice that each of the products in dispute raises scientific and technical issues that are specific to the particular construct and genetic modification that has been engineered in each of them, and to their intentional or unintentional use.

28. The generic trait (or traits) such as herbicide resistance, or disease resistance, introduced by genetic modification in the GM products in dispute may relate to a common "basic" set of generic issues (which may even be sometimes similar to some issues raised by non genetically engineered products). But it is also very clear from the experts replies that each product's specific characteristics (e.g.: glufosinate or glyphosate resistance, Bt toxin expression, or a combination of them) raises for each individual situation and product a range of new and extremely specific scientific questions. This applies for each species at stake (e.g.: sugar beet, oilseed rape, maize, potato, etc…), and has to take into account the genetic construction, the combination and the expression of these characters in each product's genetic makeup, as well as how and in which specific environment the product is going to be intentionally or unintentionally used.

29. These case-specific considerations, which cannot be automatically derived from a common set of basic and generic questions, are due in part to the fact that most of the specific characters introduced in the GM products at stake (e.g.: ARMG, glufosinate or glyphosate resistance, systemic endogenous Bt toxin expression, etc…) do not exist, cannot be obtained or have not yet been found, in non genetically modified plants. The advice before the Panel illustrates the need to take into account the international consensus that a case by case and thorough approach is justified for each and every genetically modified product. Each individual product has to be considered rigorously and assessed on the basis of its own individual merits, taking into account the relevant set of specific information pursuant to that very product, and its intentional or unintentional specific recipient environment.

C. SYSTEMIC ISSUES (INTERACTIONS AND CUMULATIVE EFFECTS)

30. The advice before the Panel also confirms that a case by case assessment may need to be supplemented by the assessment of "systemic" impacts. Such impacts are specific to the combined use of GM products in dispute, i.e. issues which are originating from potential impacts on "systems" (e.g. agricultural or agro environmental, for instance), namely potential changes induced in different "systems" by the use, in combination or in temporal sequence, of several different GM products, and which can not be identified by a case by case approach.

31. Having regard to the experts' advice, these systemic questions often open up large areas of scientific uncertainty. This is because the scientific or technical knowledge is often not complete, due to the novel character of the GM products in dispute, or the fact that the empirical and limited experience gained in one type of system (for instance in one of the three complainants' systems) cannot be transposed to a corresponding system elsewhere.

D. FAMILIARITY

32. The scientific advice provided to the Panel clearly shows, in particular in the light of the amount of scientific issues that were or are still unresolved or unclear, that some issues have not yet been studied at all, that the products in dispute have regularly given rise to many new questions in the last ten years, and that they remain at the forefront of scientific knowledge, both in terms of risks and benefits. The Communities considers that the lack of familiarity with the issues raised must be taken into account by the Panel in understanding the way new problems and corresponding requests for further information have arisen over time, in the face of developing scientific understanding.

E. EVOLVING SCIENCE

33. It is also striking that the experts confirm how little was known on so many of the relevant issues only 10 to 15 years ago, and how much the scientific understanding of many of these issues has developed since then, including in international fora such as the Codex. Developments identified by

the experts include the identification of previously unsuspected areas of risks and impacts, or identification of flaws in the way risk assessments may have been conducted in the past, both issues which are still moving very fast forward today.

34. One of the explanations for the evolution of the scientific understanding of the relevant issues before the Panel is that the sience, by being confronted with the necessity to address such new complex impacts and networks of interactions, for which no or little data was available, had to make necessary shortcuts and to take a reductionist approach. It thereby initiated the process of assessing the relevance and likelihood of some potential impacts that were first identified, but as a consequence of the shortcuts and the necessary reductionism of the scientific process, it largely overlooked many issues which are now on the table in most countries confronted with the GM products in dispute, including the three complainants. This has a major consequence, which is that a scientific risk assessment opinion is only to be considered relevant at a certain point in time, within the context of the specific impacts it had looked at, and within the context of the scientific considerations and methodologies that were available to it.

F. SCIENTIFIC EVIDENCE OF RISKS OR ABSENCE OF RISKS

35. As noted by Dr. Andow and Dr. Squire, an absence of scientific evidence does not constitute evidence of an absence of impacts or risks. This is in particular important in the case of these novel products, when a relevant new scientific issue, raised in the context of these GM products' assessments, has even not yet been studied, or not studied with proper scientific methodologies. Even though science cannot be asked to prove an absence of risk or a negative result, the novel character of the products in dispute and the issues they raise justifies the requests for new scientific information, in a context where scientific understanding is constantly evolving and new issues of potential impacts of GM products are continuously arising.

G. CONTROVERSY

36. In the limited areas of expertise in which there is overlap between the Panel's experts, or where independent scientific opinion has been sought by the European Communities on these expert replies, it is noteworthy that there is extensive disagreement between the experts or with independent scientists. This indicates a clear lack of consensus in the scientific circles on the issues at stake in these proceedings. This lack of consensus is partly explained by the questions addressed below. The European Communities considers that the Panel cannot make definitive findings of fact in the face of such conflict of views or uncertainty, beyond indicating the existence of such differences or uncertainty.

H. ABSENCE OF AGREED SCIENTIFIC CRITERIA

37. As stated by Dr. Squire in his general notes on ecological and environmental standards, one of the problems in addressing issues of science in these proceedings, is the absence of agreed criteria on many issues (in scientific and regulatory circles), including in respect of the information necessary to perform a risk assessment and, also, the manner in which to interpret the relevant data. The novel character of many of the issues at stake, which often emerged only within the past decade, is largely responsible for the absence of agreed criteria. This contributes to further scientific uncertainty, in particular as regards the impacts on the environment and the agro-ecosystem at large.

I. JUDGING WHEN THE SCIENTIFIC INFORMATION IS SUFFICIENT

38. It is apparent from the scientific advice now before the Panel, that there is no unique, absolute, scientific cut off threshold available to decide whether a GM product is safe or not (the risk

assessment end point). From the experts opinion it appears that there is a general trend, as the scientific data accumulate, that indicates a move towards the formulation of a clearer scientific opinion. However, to decide when the available data is sufficient to conclude that an element of the risk assessment is adequate or not still involves the judgement of experts (ideally this should be expertise which is independent of the regulatory authorities and the legislator, as recommended by the Codex guidance on risk analysis). More importantly, experts may disagree on whether there is a sufficiency of information (as they do in the present case very frequently) without necessarily disagreeing on the science. There may be a legitimate difference of view not on what is the state of scientific knowledge, but how it is to be applied to a particular set of facts. It is clear that, expert judgement and subjectivity are important elements of a science based risk assessment. In this regard, it appears rather clearly from Dr. Healy's considerations that she often believes that the information available for a specific dossier may have been sufficient to reach a positive conclusion; yet she may still agree remaining uncertainties exist and have been identified. In her approach these are of low significance – she may be less cautious (or precautionary) than another expert -, while other experts, on the same data, may have an equally valid judgement and reach a different conclusion as to the consequences to be drawn from such uncertainties. They may, for example, conclude that further information is necessary to try eliminate uncertainties to a greater extent.

J. INTERPRETATION OF SCIENTIFIC INFORMATION

39. Another source of the experts divergent conclusions derives from differences in data interpretation – disagreements on the science. Differences of individual experts' arguments and approaches leading to their scientific advice, which is sometimes conflicting despite being faced with the same sets of scientific facts and data, illustrates the subjective character of the Panel's experts' and other scientists' opinions in this field. This indicates that the scientific data at stake requires a substantial amount of scientific interpretation. It also confirms that the novel character of these GM products and the new issues they raise may require a different approach, and that any interpretation cannot be based on agreed criteria and methodologies or on a non-existent scientific consensus.

K. AVAILABILITY OF RISK MITIGATION AND RISK MANAGEMENT TOOLS

40. There are two general points stemming from the experts' advice that merit attention in relation to risk assessment and risk management in the context of availability of mitigation and monitoring tools. First, as for the science, it appears clearly from the experts advice that the availability and appropriateness of mitigation measures has evolved over time (particularly since 1998) in parallel with the evolution of scientific understanding of the issues. Even if mitigation measures may be available today for many of the potential impacts at stake, they are not available for all of them, and they were not available only a few years back. Furthermore, even when they have been available for some time, it has often been necessary to revise them as evolving science identified them as being inappropriate.

41. Second, it is apparent that the introduction of any GM product into the environment may cause an irreversible effect under certain conditions, in particular if a particular product, or a particular genetic modification, has the ability to maintain or spread itself into the environment. Therefore, if uncertainties remain for a particular product that may have these characteristics, any available mitigation measure may be considered on policy grounds to be inadequate. This is especially the case if the effects are irreversible.

L. POST MARKET MONITORING AND GENERAL SURVEILLANCE

42. Even if there is a degree of consensus among the Panel's experts as regards the necessity for post market monitoring of potential adverse impacts, they sometimes refer only to the monitoring of risks identified in the process of risk assessment.

43. A number of the experts indicate that there might be no need for monitoring if a particular risk has not been identified. The European Communities disagrees with this view. It considers that it may be necessary, in the face of the lack of familiarity with the new GM products at stake, to implement monitoring plans as a general post marketing surveillance tool to identify *unexpected* effects. This is consistent with international instruments. As scientific understanding of the issues has evolved, the discovery of new potential impacts that had not been thought of, or identified in earlier risk assessments, is an ample demonstration of the validity of such approach. Dr. Andow, Dr. Snow and Dr. Squire agree that some ecological or agro-environmental hazards predicted in the late 1990's are only beginning to emerge now. This is the case for the molecular basis of the transformation, horizontal gene flow, Bt and herbicide resistance management, and non target species.

44. Furthermore, if a risk is found to be of limited or no impact in an expert opinion, it is still appropriate to consider the availability of general surveillance and specific monitoring plan to check the validity of that expert opinion, and to ensure that practices do not lead to more harmful outcomes than anticipated. This may be the case, for example, when monitoring the impact of the occurrence, maintenance or spread of occasional escapes from non cultivated crops with high weediness potential, such as oilseed rape. In this respect, the European Communities would like to draw the attention of the Panel to the latest international expert advice provided to the FAO in this field, which has recommended that *any* responsible deployment of genetically modified crops needs to comprise the whole technology development process, from pre-release risk assessment to post release monitoring.[1]

M. SURVEILLANCE AND FOOD SAFETY

45. As regards food safety, even if some GM products have been found to be safe and approved on a large scale, for instance by the three complainants, the lack of general surveillance and consequently of any exposure data and assessment, means that there is no data whatsoever available on the consumption of these products – who has eaten what and when. Consequently, one can accept with a high degree of confidence that there is no acute toxicological risk posed by the relevant products, as this would probably not have gone undetected – even if one cannot rule out completely acute anaphylactic exceptional episodes. However, in the absence of exposure data in respect of chronic conditions that are common, such as allergy and cancer, there simply is no way of ascertaining whether the introduction of GM products has had any other effect on human health.

N. REMOVING UNCERTAINTIES WITH LARGE SCALE CULTIVATION

46. Some of the opinions put forward by the experts address the need to collect further information, through large scale cultivation, in order to remove some remaining scientific uncertainties. They touch in particular on large scale impacts on biodiversity and the agro-ecosystems, in the light of the recently available information on these issues. The European Communities considers that it may be appropriate to request further information on the relevant issues prior to the grant of the relevant authorization to cultivate the product. Another regulatory regime, dealing with consents for deliberate releases of genetically modified organisms for purposes other than placing on

[1] FAO expert consultation on monitoring the environmental effects of GM crops. Available at: <http://www.fao.org/newsroom/en/news/2005/89259/index.html>.

the market,[2] has already enabled several such studies to be conducted (see in particular the Farm Scale Evaluation studies). Other such studies are currently under way.

O. DETECTION METHODS

47. On the availability of detection methods, and their relationship with risk assessment and risk management, some of the Panel's experts seem to indicate that they are not related to the risk assessment process, and hence, with the risk management process, and that they are only available for the purpose of identifying individual products.

48. The European Communities would like to make clear its view that detection methods, including event-specific and quantitative methods, are necessary for the purpose of risk management, even in the absence of an identified risk in the process of risk assessment. First, and independently of any other identification purposes, they are necessary to ensure compliance and control of compliance with the legislative framework that provides for the risk assessment (only products satisfying specific legislative criteria for assessment and consents, positive opinion of advisory bodies, and/or specific thresholds of presence, may be found to be present). This requires the help of these quantitative, event-specific, detection methods.

49. Second, they may be necessary to enable the withdrawal of a specific product from the market. This could be in case of the emergence or occurrence of a previously unanticipated risk, or in case of new uncertainties as regards some significant risks that still need to be assessed, or in case of illegal placing on the market of a product. The two latter conditions occurred in the United States when the Starlink product had to be withdrawn from the food and feed chains a few years ago, even when found only in minute traces.

P. NEW DEVELOPMENTS OF RISK ASSESSMENT CONCEPTS FOR GENETICALLY MODIFIED PRODUCTS

50. The development of risk assessment concepts in the last 10 to 15 years reflected the progress in the understanding of unintended effects of GM products, and of biotechnological methods in plant breeding. Experiences drawn from a growing body of risk assessment processes, for different GM products, often indicate similar underlying problems. Early regulations (e.g. Directive 90/220/EEC) for genetically modified organisms did not differentiate between systemic environmental and product specific risks assessments, whereas most modern regulations differentiate between a general environmental assessment and specific assessments for specific products, such as GM plants for pharmaceuticals, foods and/or feeds, seeds, chemicals or even fibre products. Consequently, specific risk assessment procedures were or are currently being developed for these products. These new specifications resulted in a diversification for the risk assessments. However, experience drawn from the accumulated evidence identified several underlying problems, most notably the need for a molecular (genotypic) and phenotypic characterisation and assessment of potential unintended molecular effects was identified as the basis for the assessment in all fields.[3]

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[2] "Part B" or Directives 90/220/EEC and 2001/18/EC.

[3] Andow, D.A., Hilbeck, A., 2004: Science-based risk assessment for non-target effects of transgenic crops. – BioScience 54(7): 637-649. FAO/WHO, Safety assessment of foods derived from genetically modified animals, including fish, a joint FAO/WHO expert consultation on food derived from biotechnology, Rome, Italy, 17-21 November 2003. Available at <http://www.who.int/foodsafety/biotech/meetings/en/gmanimal_reportnov03_en.pdf>.

1. The "substantial equivalence concept"

51. The concept that a comparison of a final product with one having an acceptable standard of safety would provide an important element of safety assessments of GMOs was a commonly used basis for the development of both food safety and environmental risk assessment.[4] This concept was elaborated by FAO, WHO and OECD in the early 1990s and referred to as 'substantial equivalence' for the assessment of GM foods. But already in 2000 an FAO/WHO consultation acknowledged that this concept of substantial equivalence had attracted criticism from the perception that it was the end-point of a safety assessment rather than being the starting point.[5] The consultation concluded that consideration of compositional changes is not the sole basis for a valid determination of safety and that safety can only be determined when the results of all necessary aspects under comparison, and not merely comparisons of key constituents, are integrated. Recently, the concept has further evolved to the Comparative Safety Assessment for GMO foods.[6] By 2003/2004, most international frameworks and guidance covering living modified organisms became effective, notably on environmental safety (Cartagena Protocol on Biosafety), on food safety (Codex) and on plant health safety of genetically modified products; with all systems being based on the concept of a case by case approach. Most recently, the need for a very comprehensive molecular characterisation of each transformation event, including the analysis of integrated constructs and the flanking region, as well as the need to address potential unintended effects, was appreciated for food safety and environmental assessments.[7] These ideas were also reinforced by several general recommendations of expert advice, such as for instance a United States EPA FIFRA recent expert panel.[8]

2. Codex principles for the risk assessment of GM foods

52. The *Codex Alimentarius Commission* adopted the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, and the draft Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants and Microorganisms in 2003.[9] The Principles for the Risk Analysis – sets out important principles, such as those related to whole food

[4] WHO, 1991. Strategies for Assessing the Safety of Foods Produced by Biotechnology, Report of A Joint FAO/WHO Consultation. World Health Organisation, Geneva. Available at <http://www.who.int/foodsafety/publications/biotech/1990/en/>.

[5] FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, 29 May - 2 June 2000. Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/es/esn/food/gmreport.pdf; Millstone, E., Brunner, E., Mayer, S., 1999. Beyond 'substantial equivalence'. Nature 401, 525-526; Schenkelaars, P., 2002. Rethinking substantial equivalence. Nature Biotechnology 20, 2, 119.

[6] Kok, E.J., Kuiper, H.A., 2003. Comparative safety assessment for biotech crops. Trends in Biotechnology 21, 439-444.

[7] FAO/WHO, Safety assessment of foods derived from genetically modified animals, including fish, a joint FAO/WHO expert consultation on food derived from biotechnology, Rome, Italy, 17 - 21 November 2003. Available at <http://www.who.int/foodsafety/biotech/meetings/en/gmanimal_reportnov03_en.pdf>.

[8] SAP Report No. 2004-05. MINUTES of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) Meeting, June 8-10, 2004, Arlington, Virginia. A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management For Bacillus thuringiensis (Bt) Cotton Products. 84 pages.

[9] Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; CAC/GL 44-2003; Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; CAC/GL 45-2003; Guideline for the Conduct of Food Safety Assessment of Foods Produced using Recombinant-)DNA Microorganisms; CAC/GL 46-2003. Codex Alimentarius Commission, 2003. These were provided to the Panel by the European Communities as part of Exhibit EC-44 to the first written submission. Available at <ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf>.

assessment, uncertainties, sustainability of data provided to the risk assessment, and review of current risk assessment processes.

53. Common agreed principles for the safety assessment dictate a case-by-case pre-market assessment on the basis of a Comparative Safety Assessment (CSA). The CSA is basically a two-tiered approach. The initial step is comprised of a thorough comparison (not limited to a simple comparison of major key nutrients or phenotypes) with the closely related conventional food organism counterpart (there is however continuous debate as to what may constitute a suitable control counterpart), to identify any differences that may have safety implications for the consumer. This comparison includes both thorough phenotypic characteristics as well as a thorough compositional analysis (primary and secondary metabolites known to be bio-active). The second step comprises the toxicological and nutritional evaluation of the identified differences between the food derived from the GMO and its comparator. Hazard identification and characterization are typically the first steps in any risk assessment, and an extensive molecular characterisation of the inserted genetic material construct is required as a prerequisite to enable a sound hazard identification and characterization.

54. The safety of newly introduced gene products must be assessed on a case-by-case basis. Following the phase of hazard identification, characterization and food intake-assessment (including, where necessary, target animal and/or whole feed studies), an integrated toxicological evaluation (potentially covering both acute and chronic exposures) will combine all the information with relation to the food safety of the GMO-derived food.

55. As is discussed elsewhere (see discussion in our comments on Q111), genetic modification may induce dramatic unintended changes in the composition of foods, which may have gone undetected if only key nutriments comparison had been performed.[10] For the identification of potentially occurring unintended effects, metabolic profiling methods have been proposed, and different possibilities for profiling methods have been characterized.[11] In addition to investigating health risks directly associated with food production, the Codex risk assessment has and is still broadening to include indirect effects, and now also encompasses effects of new products on the environment that may have an indirect impact on human health.[12]

3. The environmental safety assessment of GMOs

56. A case by case assessment considering each GM organism derived from a single transformation event, or from each combination of them, as well as considering the different receiving environments (intentionally or unintentionally), is broadly recognised as the best basic framework for assessing environmental risks of GMOs. Internationally, in particular within the OECD, a concept of familiarity was developed also in the framework of environmental safety of transgenic plants. The concept may facilitate risk assessments, because achieving familiarity means having enough and thorough information, in order to then be able to make a sound and experienced judgement on safety or risk.[13] Unfortunately, as has been shown eloquently by the experts' replies, there is still

[10] See, for instance, Metabolic Profiling Allows Comprehensive Phenotyping of Genetically or Environmentally Modified Plant Systems, Ute Roessner, Alexander Luedemann, Doreen Brust, Oliver Fiehn, Thomas Linke, Lothar Willmitzer, and Alisdair R. Fernie; The Plant Cell, Vol. 13, 11–29, January 2001.

[11] Kuiper, H.A., Kok, E.J., Engel, K.H. (2003) Exploitation of molecular profiling techniques for GM food safety assessment. Current Opinion in Biotechnology 14, 238-243.

[12] Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741.

[13] NAS (National Academy of Sciences) (2002) Environmental Effects of Transgenic Plants:the Scope and Adequacy of Regulation. Washington, DC: National Academy Press http://books.nap.edu/books/0309082633/html/index.html. Conner, A.J., Glare, T.R. and Nap.J.P. 2003. The

considerable lack of familiarity with many ecological systems and interactions occurring both in cultivated and natural environments.

57. Familiarity could then also be used to indicate appropriate management practices, including the evaluation whether standard agricultural practices are adequate or other management practices are needed to manage the risk.[14] Familiarity depends also on having existing knowledge on the range of environments in Europe, their agro-ecosystems, and the relevant ecological webs, in order to determine the interaction of introduced organisms with these environments and to conduct the risk assessments. In addition, risk assessments will need to consider that agricultural practices in one specific geographical regions, may differ from that in another region. Furthermore, as discussed above and in the relevant experts' replies, there are also broader systemic agro-environmental risk issues which need to be assessed on top of each individual case by case assessment for a specific organism, in a given environment. For example, to assess impacts of GM crops and associated management practices on soil ecosystems it is now considered more appropriate to measure changes in soil ecological functions (e.g. soil nutrient cycling) rather than monitor an individual indicator species.[15]

58. Currently, the Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity is the only international regulatory instrument which addresses specifically the potential adverse effects of GMOs on "the conservation and the sustainable use of biological diversity", taking also into account effects on human health.[16] This instrument calls for scientifically sound risk assessments, and the establishment of appropriate risk management measures to prevent adverse effects of living modified organisms (LMOs) on the conservation and the sustainable use of biological diversity, taking also into account risks to human health. In doing so, it provides in its Annex III for a general set of risk assessment principles, methodology, steps and points to consider. However, it should be noted that the development of detailed guidance on risk assessment and risk management of LMOs will only happen in the future, as it is currently on the agenda of the next meetings of the parties to the CPB.

59. Annex III of the CPB focuses especially on identification of any novel genotypic and phenotypic characteristics that may have adverse effects on biological diversity in the likely potential receiving environment. The information on the receiving environment includes data on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of species contained within the likely potential receiving environment (region or country). As the focus of the CPB is biodiversity, its consideration of human health safety is limited, concentrating on situations in which the LMO itself may end up in the food supply, such as might happen via trade of crop seeds, or as regards indirect human health effects that may arise from direct impact of the GM crop and/or its management system on local biodiversity.

60. Recent work for the implementation of the CPB recommends, as regards the assessment of non target environmental risks, the analysis of effects of GMOs on important indicator species in the environment, before an assessment of effects on the biodiversity. This is mainly because a better access is available to species assessment and there are serious methodological limitations for an

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release of genetically modified crops into the Environment. The Plant Journal 33, 19–46. Poppy & Sutherland 2004; Physiol Ent 29: 257-268.

[14] OECD 1993 SAFETY EVALUATION OF FOODS DERIVED BY MODERN BIOTECHNOLOGY, CONCEPTS AND PRINCIPLES. Available at <http://www.oecd.org/dataoecd/57/3/1946129.pdf>.

[15] Birch et al., 2004 In: Environmental Risk Assessment of Genetically Modified Organisms vol 1. a case study of Bt maize in Kenya. Eds Hilbeck and Andow. CABI Publishing. pp 117-1850.

[16] See the discussion in the first written submission of the European Communities, paras 87 to 112.

analysis of effects to biodiversity and the diversity of species in general.[17] Specifically, a non target individual species risk assessment, for assessing the non target effects of GM crops, was considered to be more useful than the conventionally used eco-toxicology or non-indigenous-species model: this new ecological risk assessment model requires information about the intended receiving environment and aims to focus on the analysis of possible effects of a GMO on local species which are assessed as having a specific and regionally-important ecological function in this system or have special conservation or heritage value to the country.

61. However, toxicity tests are obviously considerably more difficult to conduct with endangered species. Where, for instance, native insects may have particular cultural value, studies to understand their distribution and ecology in agricultural habitats, and methods for conducting laboratory bioassays with them, may help with GM plant risk assessment.[18] However, not all species are amenable to such bioassays, because they are often protected.

4. The plant health assessment of GMOs

62. Recently, within the framework of the International Plant Protection Convention, (hereinafter "IPPC") guidance on the plant health risk assessment of LMOs has been developed in harmony and mutual supportiveness with the principles of the CPB. This guidance (ISPM11), provides a useful and detailed guidance to address essentially plant pest risks (risks to plant health) that may arise from the GM product itself. It is noteworthy, however, that the framework of the IPPC, although addressing plant health in general, has focused its technical expertise and work on crop plant health, rather that on health of any wild plant health to be found in the ecosystem, nor on the ecological food webs involving non cropped wild plants to be found in natural ecosystems in the same region as the GM crop.

5. Starting points for assessing effects of GMOs on food safety, mediated through the environment

63. Both the Codex principles for food safety and the risk assessment provided for by the CPB, provide opportunities to more explicitly consider interactions between food safety, feed safety and environmental safety. For instance, the broadening of the Codex risk assessment to include indirect effects, provides for assessing effects on the environment that may have an indirect impact on human health.[19]

64. Indirect effects on human health or the environment may be described as effects occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management associated with the GM crop in the region or country of use.[20] Indirect effects are likely to be delayed and possibly involve sub-chronic, long term effects. Given examples include impacts which can arise from changed agricultural practices associated with the management of a genetically modified (GM) crop rather than directly from the genetically modified crop itself. The above mentioned explanatory guide suggests that

[17] Andow & Hilbeck (2004). Science-based risk assessment for non-target effects of transgenic crops. BioScience 54: 637-649. Birch et al., 2004 In: Environmental Risk Assessment of Genetically Modified Organisms vol 1. a case study of Bt maize in Kenya. Eds Hilbeck and Andow. CABI Publishing. pp 117-1850.

[18] O'Callaghan, Glare, Burgess and Malone (2005). Effects of plants genetically modified for insect resistance on nontarget organisms. Annu. Rev. Entomol. 50: 271-292.

[19] Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741.

[20] CPB, explanatory guide. Available at <http://www.biodiv.org/doc/books/2003/B-01669.pdf> (last visited on 31 January 2005).

questions related to human health effects cover the human health aspects that are linked to the state of use and conservation of biological diversity, or the environment in general. Such a direct link exists if the health effect is consequent to an exposure to the risk *in situ*, for instance, if a farmer were to develop an allergenic reaction to pollen from genetically modified plants; but it also exists indirectly if the health effect resulted from effects or changes on biological diversity (indirect or secondary effect). Direct effects on human health (e.g. caused by consumption of GM food, or exposure to pharmaceuticals for humans or animals) would, however, be mainly covered by human health focused instruments (Codex, WHO, OIE).

6. The animal health assessment of GMOs

65. It is finally critical to note that, as regards animal health impacts of GM plant or other GM products (impacts on animals used for food, breeding animals, or even on all relevant non domesticated organisms from the animal kingdom), or as regards target or non target animal feed safety, there is not yet much specific risk assessment guidance developed by international expert consultation or organisations. It is important to consider that the lack of identification of human health risks in the risk assessment of a GM plant used as food, does not necessarily correlate with an absence of risks on target or non target animals in the framework of feed safety, in particular for non mammals. This relates to, among others, differences in animal physiology and metabolism, parts/products of crop plants that are consumed, processing, and exposure (intake) levels. As has been indicated by the OIE in its recent submission, the OIE's international standards are not linked directly with modern biotechnology. Consequently, indirect environmental or human health effect that may arise from direct impacts on animal health or GM plant induced imbalance in the animal interactions with the ecosystem is still a largely an unexplored area.

Q. OTHER GENERAL ISSUES – DEALT WITH IN THE COMMENTS ON THE QUESTIONS

66. There are a number of other general issues that arise from the advice provided to the Panel that the European Communities discusses in commenting on the questions to which they relate, as identified below in brackets after each issue. These include:

- Unintended effects, and general molecular aspects of genetic modification, and transgenics (Q111);
- Molecular characterization requirements (Q9);
- Correlation between molecular characterization and toxicological assessment, according to substantial equivalence and comparative assessments (Q9 and Q111);
- Allergenicity assessment (Q111);
- Horizontal gene transfer (Q1 and Q2);
- Cross breeding and assessment of stacking of genetic modification (Q111);

IV. THE PANEL'S GENERAL QUESTIONS (N°S 1 TO 9 AND 110 TO 114)

Question 1

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that antibiotic resistance marker genes (ARMG) pass from a biotech food or feed product to bacteria or other micro-organisms present in the human or animal gut?

(a) *If scientific evidence indicates that such an event could occur, what risks, if any, would arise from that event? What is the comparative relevance or magnitude of this risk in relation to the likelihood of such a transfer from other sources of antibiotic resistance not involving the use of recombinant DNA technology?*

(b) *If such risks have been identified, what is the likelihood of adverse effects to human or animal health, in light of the processing of raw biotech products into human food or animal feedstuffs?*

(c) *Are these consequences relevant to the specific types of ARMG currently used in the products at issue in this dispute? Please explain.*

(d) *If such risks have been identified, what risk management options are available to mitigate those risks and what is their efficacy?*

General comments

67. The Panel's first general question sought advice on the issue of whether, how, and what are the consequences if antibiotic resistance marker genes (ARMG) could pass from GM food or feed product to bacteria or other micro-organisms present in the human or animal gut. However, it sought also in its next question equally important advice as to whether other routes than food or feed, and persistence into the environment and in soil of plant DNA, may also lead to the development of antibiotic resistance.

68. Unfortunately only one of the Panel's experts, Dr. Nutti, has addressed the issue of ARMG, and then has limited her comments to question 1 with the exception of sub-questions c) and d). Also, Dr. Nutti does not respond to question 2, stating that it is a question relating to environmental safety on which she does not have expertise.

69. Dr. Nutti's response is however characterised by a lack of detailed arguments and failure to cite and discuss the most relevant and updated scientific evidence, and also presents an erroneous conclusion. It therefore is seriously misleading to those unfamiliar with the true state of scientific knowledge and understanding. Dr. Nutti is definitively not correct in her statement that

> To date, there are no reports that marker genes in plant DNA transfer to these (microbial and mammalian) cells.

70. She is equally seriously misleading by not citing the most recent and relevant Codex guidance as regards ARMG.

71. The only other contribution that Dr. Nutti makes is to seek to minimise with sweeping statements the adverse consequences that would be caused by a transfer of antibiotic resistance. This is again very superficial, and she does not answer all parts of the Panel's question 1.

72. Because of the inadequacy, incorrectness and incompleteness of the advice provided to the Panel on related Questions 1 and 2, the European Communities provides its own analysis below on each question. The European Communities has prepared this information with the assistance of

scientists, experts in the field of antibiotic resistance and ARMG, and presents it as objectively as possible so as to complete the information available to the Panel.[21]

73. On the basis of this analysis, it can be concluded that:

– The demonstration has been made that antibiotic resistance genes from GM plants can be captured and expressed by bacteria, including pathogenic bacteria.

– Based only on the few studies that exist, the occurrence in nature of such an event is considered as low but not zero.

[21] The following are the complete references relating to the discussion in this section:

Bertolla F, Kay E, Simonet P. Potential dissemination of antibiotic resistance genes from transgenic plants to microorganisms. Infect Control Hosp Epidemiol. 2000, 21:390-3.

Coelho J, Woodford N, Turton J, Livermore DM. Multiresistant Acinetobacter in the UK: how big a threat? J Hosp Infect. 2004, 58:167-9.

de Vries J, Heine M, Harms K, Wackernagel W. Spread of recombinant DNA by roots and pollen of transgenic potato plants, identified by highly specific biomonitoring using natural transformation of an Acinetobacter sp. Appl Environ Microbiol. 2003,69:4455-62.

de Vries J, Herzfeld T, Wackernagel W. Transfer of plastid DNA from tobacco to the soil bacterium Acinetobacter sp. by natural transformation. Mol Microbiol. 2004;53:323-34.

de Vries J, Wackernagel W. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proc Natl Acad Sci U S A. 2002, 99:2094-9.

Droge M, Puhler A, Selbitschka W. Horizontal gene transfer as a biosafety issue: a natural phenomenon of public concern. J Biotechnol. 1998, 64:75-90.

Duggan PS, Chambers PA, Heritage J, Forbes JM. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiol Lett. 2000, 191:71-7.

Duggan PS, Chambers PA, Heritage J, Michael Forbes J. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. Br J Nutr. 2003, 89:159-66.

Gebhard F, Smalla K. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol Ecol 1999, 28;261-72.

Gebhard F, Smalla K. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol. 1998, 64:1550-4.

Gordon KA, Jones RN; SENTRY Participant Groups (Europe, Latin America, North America). Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: comparison report to Europe and Latin America. Results from the SENTRY Antimicrobial Surveillance Program (2000). Diagn Microbiol Infect Dis. 2003, 45:295-301.

Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother. 2004,38:1449-59.

Kay E, Vogel TM, Bertolla F, Nalin R, Simonet P. In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Appl Environ Microbiol. 2002, 68:3345-51.

Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA, Flint HJ. Fate of free DNA and transformation of the oral bacterium Streptococcus gordonii DL1 by plasmid DNA in human saliva. Appl Environ Microbiol. 1999, 65:6-10.

Mercer DK, Scott KP, Melville CM, Glover LA, Flint HJ. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. FEMS Microbiol Lett. 2001,200:163-7.

Nielsen KM, Bones AM, Smalla K, van Elsas JD. Horizontal gene transfer from transgenic plants to terrestrial bacteria--a rare event? FEMS Microbiol Rev. 1998, 22:79-103.

Nielsen KM, van Elsas JD, Smalla K. Transformation of Acinetobacter sp. strain BD413(pFG4DeltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Appl Environ Microbiol. 2000, 66:1237-42.

Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal 2004:48;1-18

Paget E, Simonet P. On the track of natural transformation in soil. FEMS Microbiol Ecol 1994, 15:109-18.

– The risk for human health should be analysed case by case. The kanamycin resistance gene presents the lowest level of risk, the ampicillin and streptomycin/spectinomycin resistance genes present a higher degree of risk (although still limited). In particular, the fact that the resistance genes are already naturally present limits the risk.

Detailed comments

74. Dr. Nutti refers the Panel to an old FAO/WHO expert consultation from 2000 to state that DNA transfer from food derived from plants to microbial or mammal cells, under normal circumstances of dietary exposure, would require all the following events to occur:

– the relevant gene(s) would have to be released, probably as a linear fragment;
– the gene(s) would have to survive nucleases in the plant and in the gastrointestinal tract;
– the gene(s) would have to compete for uptake with dietary DNA;
– the recipient bacteria or mammalian cells would have to be competent for transformation
– and the gene(s) would have to survive their restriction enzymes; and
– the gene(s) would have to be inserted in the host DNA by rare repair or recombinant events.

75. This citation may be correct, but Dr. Nutti omits to cite the most relevant and recent Codex guidance as regards ARMG. The Codex guidelines for the conduct of food safety assessment of foods derived from recombinant-DNA plants[22], provide detailed guidance on the use of antibiotic resistance marker genes in its paragraphs 55 to 58:

> 55. Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods should be used in the future development of recombinant-DNA plants, where such technologies are available and demonstrated to be safe.
>
> 56. Gene transfer from plants and their food products to gut microorganisms or human cells is considered a rare possibility because of the many complex and unlikely events that would need to occur consecutively. Nevertheless, the possibility of such events cannot be completely discounted.
>
> 57. In assessing safety of foods containing antibiotic resistance marker genes, the following factors should be considered:
>
> A) the clinical and veterinary use and importance of the antibiotic in question; (Certain antibiotics are the only drug available to treat some clinical conditions (e.g. vancomycin for use in treating certain staphylococcal infections). Marker genes encoding resistance to such antibiotics should not be used in recombinant-DNA plants.)
>
> B) whether the presence in food of the enzyme or protein encoded by the antibiotic resistance marker gene would compromise the therapeutic efficacy of the orally administered antibiotic; and (This assessment should provide an estimate of the amount of orally ingested antibiotic that could be degraded by the presence of the

[22] CAC/GL 45-2003.

> enzyme in food, taking into account factors such as dosage of the antibiotic, amount of enzyme likely to remain in food following exposure to digestive conditions, including neutral or alkaline stomach conditions and the need for enzyme cofactors (e.g. ATP) for enzymatic activity and estimated concentration of such factors in food.)
>
> C) safety of the gene product, as would be the case for any other expressed gene product.
>
> 58. If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in the food. Antibiotic resistance genes used in food production that encode resistance to clinically used antibiotics should not be present in foods [emphasis added, footnote omitted].

76. The Codex Guidelines clearly addresses the risk management options that should be considered, and, as will be discussed below, many of the ARMG present in the products at stake in these disputes encode resistance to antibiotics that are clinically used, even if in a limited number of pathologies, and therefore require a more careful scrutiny than advocated by Dr. Nutti.

77. Similarly, the Codex guidelines for the conduct of food safety assessment of foods produced using recombinant-DNA Micro-organisms (CAC/GL 46-2003), provide detailed guidance on antibiotic and gene transfer in its paragraphs 51 to 54:

> 51. In general, traditional strains of microorganisms developed for food processing uses have not been assessed for antibiotic resistance. Many microorganisms used in food production possess intrinsic resistance to specific antibiotics. Such properties need not exclude such strains from consideration as recipients in constructing recombinant-DNA microorganisms. However, strains in which antibiotic resistance is encoded by transmissible genetic elements should not be used where such strains or these genetic elements are present in the final food. Any indication of the presence of plasmids, transposons, and integrons containing such resistance genes should be specifically addressed.
>
> 52. Alternative technologies, demonstrated to be safe, that do not rely on antibiotic resistance marker genes in viable microorganisms present in foods should be used for selection purposes in recombinant-DNA microorganisms. In general, use of antibiotic resistance markers for constructing intermediate strains should pose no significant hazards that would exclude the use of the ultimate strains in food production, provided that the antibiotic resistance marker genes have been removed from the final construct.
>
> 53. Transfer of plasmids and genes between the resident intestinal microflora and ingested recombinant-DNA microorganisms may occur. The possibility and consequences of gene transfer from recombinant-DNA microorganisms and food products produced by recombinant-DNA microorganisms to gut microorganisms or human cells should also be considered. Transferred DNA would be unlikely to be maintained in the absence of selective pressure. Nevertheless, the possibility of such events cannot be completely discounted.
>
> 54. In order to minimize the possibility of gene transfer, the following steps should be considered:

– chromosomal integration of the inserted genetic material may be preferable to localization on a plasmid;

– where the recombinant-DNA microorganism will remain viable in the gastrointestinal tract, genes should be avoided in the genetic construct that could provide a selective advantage to recipient organisms to which the genetic material is unintentionally transferred; and

– sequences that mediate integration into other genomes should be avoided in constructing the introduced genetic material.

78. As indicated earlier, Dr. Nutti's statement that:

> To date, there are no reports that marker genes in plant DNA transfer to these (microbial and mammalian) cells.

is patently not correct, as several scientists have demonstrated such gene transfer *in natura*[23] or under simulated environmental conditions.[24] Extracts from work cited follows:

> Uptake of DNA from GM plants and transformation of micro-organisms may occur along the complete chain of events, extending from the field to food processing and storage, to the digestive tract and finally again to the environment. The highest probability of transformation events to occur can be expected when the "concentrations" of the "reactants", i.e. transformable DNA and competent bacteria are high. Highest microbial counts in a food of plant origin are found in food fermentation processes such as they occur in the production of Sauerkraut, fermented olives, tomatoes, cucumbers, egg-plants,[25] beer, sourdough, and many Asian foods based on soy, beans, peanuts, cereals or coconut. Fermentation processes are finally also important in post harvest treatment of coffee and cacao. The numbers of bacteria involved in these processes may exceed 10^9 cells per gram, and the species involved comprise bacteria from virtually all genera included in the group of lactic acid bacteria, bacilli (in Asian food), acetic acid bacteria, enterobacteria. Representative forms of many of these organisms of these groups have been shown to be transformable with DNA (Lorenz and Wackernagel, 1994)[26].
>
> Examples of the insertion of GM plant DNA into bacterial chromosomes under simulated environmental conditions have all involved highly homologous regions between an antibiotic resistance marker gene and an identical gene present in the recipient bacterial chromosome, known as marker rescue experiments.[27] The key

[23] Simonet et al, 2004.

[24] G. van den Eede, H. Aarts, H.-J. Buhk, G. Corthier, H.J. Flint, W. Hammes, B. Jacobsen, T. Midtvedt, J. van der Vossen, A. von Wright, W. Wackernagel, A. Wilcks. Food and Chemical Toxicology 42 (2004), 1127-1156.

[25] Buckenhüskes, H.J., Hammes, W.P., 1990. Starterkulturen bei der Verarbeitung von Obst und Gemüse. Bioengineering 2, 34–42. Buckenhüskes, H.J., 1993. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiology Reviews 12, 253–272.

[26] Lorenz, M.G., Wackernagel, W., 1994. Bacterial gene transfer by natural genetic transformation in the environment. Microbiological Reviews 58, 563–602.

[27] Gebhard, F., Smalla, K., 1998. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Applied and Environmental Microbiology 64, 1550–1554. Nielsen, K.M., van Elsas, J.D., Smalla, K., 2000. Transformation of Acinetobacter sp. strain BD413 (pFG4 nptII) with transgenic plant DNA in

> question must be whether such events can lead to the capture of adjacent genes flanking the region of homology. Currently we can state that this possibility does exist where the homologous region is present in a circular molecule, or where multiple regions of homology are present. Based on most recent findings in different bacterial species[28] a "homology facilitated illegitimate recombination" can increase the frequency of a basically illegitimate incorporation of genes when heterologous DNA of up to 2.9 kb is flanked by a short sequence homologous to the integration site. These findings show that the incorporation of a foreign gene and its expression is basically possible, although such an event has not yet been shown to take place in environments in which the DNA is released from the plant genome as it occurs e.g. in the intestinal tract.

79. There is now wide international agreement that horizontal transfer of genes, especially ARMG, from GM plants or products thereof to micro-organisms, especially in the gut, is unlikely, but not impossible. Therefore a case by case risk assessment is necessary. Work under the EU 5th framework project GMOBILITY has shown in *in vitro* models that transformation and integration of plasmids is obviously possible in any part of the gastrointestinal tract, and that stability of DNA from GM plants is sufficient to enable small amounts of DNA fragments to survive the passage to distal parts of the gastrointestinal tract.[29] However, no transfer could be detected in *in vivo* digestive tract models, reflecting also small likelihood. Two papers recently published in Nature Biotechnology indicate that current methods for monitoring horizontal gene transfer from transgenic crops to micro-organisms are very problematic and too insensitive to detect horizontal gene transfer. As such, even though efforts so far have largely failed to observe horizontal gene transfer in the field or have deemed frequencies too low or too rare to pose risks, this may not be true and the reality may clearly be different. The analysis by scientists from the New Zealand and Norwegian Institutes of Gene Ecology criticizes contemporary horizontal gene transfer risk assessment of transgenic crops.[30]

80. Equally, the Codex guidelines cited above indicates that gene transfer from plants and their food products to gut micro-organisms or human cells is considered a rare possibility, but that nevertheless, the possibility of such events cannot be completely discounted.

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soil microcosms and effects of kanamycin on selection of transformants. Applied and Environmental Microbiology 66, 1237–1242. de Vries, J., Wackernagel, W., 1998. Detection of nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. Molecular and General Genetics 257, 606–613.

[28] de Vries, J., Wackernagel, W., 2002. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proceedings of the National Academy of Sciences of the United States of America 99, 2094–2099. Prudhomme, M., Libante, V., Claverys, J.P., 2002. Homologous recombination at the border: insertion-deletions and the trapping of foreign DNA in Streptococcus pneumoniae. Proceedings of the National Academy of Sciences of the United States of America 99, 2100–2105. Meier, P., Wackernagel, W., 2003. Monitoring the spread of recombinant DNA from field plots with transgenic sugar beet plants by PCR and natural transformation of Pseudomonas stutzeri. Transgenic Research (in press)

[29] GMOBILITY. G. van den Eede, H. Aarts, H.-J. Buhk, G. Corthier, H.J. Flint, W. Hammes, B. Jacobsen, T. Midtvedt, J. van der Vossen, A. von Wright, W. Wackernagel, A. Wilcks. Food and Chemical Toxicology 42 (2004), 1127-1156.

[30] Heinemann JA and Traavik T. 2004 "Problems in monitoring horizontal gene transfer in field trials of transgenic plants" Nature Biotechnology 22, 1105-1109. Nielsen, K.M. and Townsend,J.P., 2004, Monitoring and modeling horizontal gene transfer. Nat.Biotechnol., 2004, 22, 9, 1110-1114.

81. More recently, the latest FAO/WHO expert consultation on foods derived from GM animals[31] agreed that the DNA construct used should be carefully considered within an assessment, since horizontal transfer or recombination may occur. Additionally, bacterial vector-derived materials may include additional sequence fragments unrelated to the target gene.[32] There was also the recognition of potential for horizontal transfer of the gene construct as food-ingested foreign DNA may not be completely degraded in the gastrointestinal tract of mice and pigs.[33] For the food safety assessment, it is therefore prudent to assume that DNA fragments may survive the human gastrointestinal tract and be absorbed by either the gut microflora or somatic cells lining the intestinal tract.

82. Several commercially available GMOs are fitted with antibiotic resistance genes which have been used as marker genes in the construction of genetically modified plants. Concerns have arisen which are of two types:

– The antibiotics administered orally in a patient could be inactivated by antibiotic resistance proteins present in food derived from a GM plants.

– The resistance genes could escape to be integrated in pathogen bacteria and expressed.

83. The first concern does not seem to be medically important on the basis that: resistance genes are generally not expressed in plants and that inactivating enzymes are for the most part inactivated in the gut. Anyway, these inactivating enzymes are also produced by resistant bacteria present as colonisers of gut, oral cavity and other mucosal surfaces and there is no clinical evidence for any impact in terms of therapy failure.

84. Only the second concern is the object of the general question 1. The European Communities will discuss below, first the detailed mechanisms and likelihood of such gene transfer, and thereafter the risks that this would pose for human (or animal) health, as well as risk management issues.

85. The transfer of cloned ARMG from genetically modified plants to pathogenic bacteria requires numerous steps (WHO/FAO, 2000, see Dr. Nutti's reply) for the process to occur under natural conditions. These may be summarized as being:

- A. DNA from GMOs has to be released from plants and to persist in an intact and active form in soils or in the gut of humans or animals fed with GM plants.
- B. DNA from GMOs present in soils or in the oral cavity of humans or animals has to penetrate into bacteria, the foreign gene has to be integrated and should be able to achieve production of resistance proteins.
- C. The foreign DNA integrated into the genome of soil bacteria or non pathogenic bacteria has to be transferred to pathogenic bacteria.

[31] FAO/WHO, Safety assessment of foods derived from genetically modified animals, including fish, a joint FAO/WHO expert consultation on food derived from biotechnology, Rome, Italy, 17 - 21 November 2003. Available at <http://www.who.int/foodsafety/biotech/meetings/en/gmanimal_reportnov03_en.pdf>.

[32] Environmental Effects of Transgenic Plants. The Scope and Adequacy of Regulation. National Research Council, 2002. National Academy Press, Washington, 320 pp.

[33] Chowdhury EH, Shimada N, Murata H, Mikami O, Sultana P, Miyazaki S, Yoshioka M, Yamanaka N, Hirai N & Nakajima Y (2003): Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves, VETERINARY AND HUMAN TOXICOLOGY 45, pp. 71-75. Schubbert et al., 1997; Schubbert et al., 1998.

A. The DNA from GMOs has to be released from plants and to persist in an intact and active form in soils or in the gut of humans or animals fed with GM plants

86. What do we know? We know that there is DNA in the soil which results from the lysis of cells. For the most part this DNA is degraded. However, a detectable quantity can still be found after a long period of time. Intact DNA of GM plant was detected by a highly sensitive molecular technique (PCR) more than two years after the harvesting of the crops[34]. The longest persistence of DNA in field soil was observed with soil from a potato field in 1997 sampled in the following year in April and then stored moist at 4 degrees C in the dark for 4 years prior to extract preparation and transformation[35]. The quality of the DNA has been testified by its capacity to penetrate into bacteria and to be integrated into their genome experimentally.

87. In the oral cavity of humans, again the DNA was rapidly degraded but a few intact DNA persisted which was demonstrated to be active[36]. Similarly, Duggan et al.[37] found that in sheep saliva and rumen fluid active DNA still persisted but was rapidly degraded in the gastro-intestinal tract. Also, intact transgenes from silage are unlikely to survive significantly in the rumen whereas DNA in maize grains persists for a significant time and may, therefore, provide a source of transforming DNA in the rumen[38].

88. In conclusion therefore, there is convincing evidence that DNA sufficiently intact to penetrate into bacteria can persist at low levels in the soil, in the oral cavity of humans and animals but that DNA is nearly completely destroyed in the lower intestinal tract.

B. The DNA from GMOs present in soils or in the oral cavity of humans or animals has to penetrate into bacteria, the foreign gene has to be integrated and should be able to achieve production of resistance proteins

89. The way of penetration of extra-cellular DNA into bacteria is called "natural transformation". Only a limited number of bacteria have been identified (nearly 40 species) which have the cellular machinery to be transformed. These species include a few pathogenic or non pathogenic (commensal) bacterial species which are usual hosts of the oral cavity of humans and animals (*Streptococcus*,

[34] Paget E, Simonet P. On the track of natural transformation in soil. FEMS Microbiol Ecol 1994, 15:109-18. Gebhard F, Smalla K. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. FEMS Microbiol Ecol 1999, 28;261-72. Gebhard F, Smalla K. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol. 1998, 64:1550-4. Kay E, Vogel TM, Bertolla F, Nalin R, Simonet P. In situ transfer of antibiotic resistance genes from transgenic (transplastomic) tobacco plants to bacteria. Appl Environ Microbiol. 2002, 68:3345-51.

[35] de Vries J, Heine M, Harms K, Wackernagel W. Spread of recombinant DNA by roots and pollen of transgenic potato plants,identified by highly specific biomonitoring using natural transformation of an Acinetobacter sp. Appl Environ Microbiol. 2003,69:4455-62.

[36] Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA, Flint HJ. Fate of free DNA and transformation of the oral bacterium Streptococcus gordonii DL1 by plasmid DNA in human saliva. Appl Environ Microbiol. 1999, 65:6-10. Mercer DK, Scott KP, Melville CM, Glover LA, Flint HJ. Transformation of an oral bacterium via chromosomal integration of free DNA in the presence of human saliva. FEMS Microbiol Lett. 2001,200:163-7.

[37] Duggan PS, Chambers PA, Heritage J, Forbes JM. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. FEMS Microbiol Lett. 2000, 191:71-7.

[38] Duggan PS, Chambers PA, Heritage J, Michael Forbes J. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. Br J Nutr. 2003, 89:159-66.

Neissseria, *Haemophilus*, and *Moraxella*)[39]; the others are present in the environment. In addition, the ability to be transformed depends on favourable environmental circumstances which are not the same according to the bacteria. Also, the incoming DNA should not be degraded by the bacteria and it is considered that integration in the chromosome by a process called recombination requires that the foreign DNA has similarity within a certain length with the host chromosomal DNA[40]. The required length of similarity depends on the bacterial species.

90. Despite these difficulties, authors succeeded in transforming *Acinetobacter calcoaceticus* (an environmental bacteria) in vitro and in soil with plant DNA bearing an antibiotic resistance gene (kanamycin or streptomycin/spectinomycin resistance)[41]. The frequencies of transformation were relatively low but variable depending on the conditions. The three important observations are:

- 1/ there was integration of the DNA (recombination) even in regions of the host chromosome with few similarity with the foreign DNA; only the presence of few and short "hotspots" of similarity were sufficient
- 2/ the integrated DNA segments often encompassed plant genes which were adjacent to the antibiotic resistance gene and
- 3/ the bacteria expressed the plant gene.

91. However, most experiments were carried out *in vitro* and a major factor limiting transformation of genes from transgenic plants is the acquisition of a state for the bacteria where it is naturally transformable. The soil conditions are probably not optimal and the frequency of transformation is very low under natural conditions[42]. However, it should be mentioned that evidence that plant genes (from natural, "non modified" plants) were transferred during evolution from plants to bacteria was demonstrated by nucleotide and protein sequences[43].

92. Therefore, it may be concluded that:

- 1/ integration of resistance genes from a plant DNA into the genome of a soil bacteria by natural transformation is possible at a low frequency but more easily than initially thought, and

[39] Reviewed in Bertolla F, Kay E, Simonet P. Potential dissemination of antibiotic resistance genes from transgenic plants to microorganisms. Infect Control Hosp Epidemiol. 2000, 21:390-3.

[40] Ibidem.

[41] Gebhard F, Smalla K. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol. 1998, 64:1550-4. Nielsen KM, Bones AM, Smalla K, van Elsas JD. Horizontal gene transfer from transgenic plants to terrestrial bacteria--a rare event? FEMS Microbiol Rev. 1998, 22:79-103. Nielsen KM, van Elsas JD, Smalla K. Transformation of Acinetobacter sp. strain BD413(pFG4DeltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Appl Environ Microbiol. 2000, 66:1237-42. de Vries J, Herzfeld T, Wackernagel W. Transfer of plastid DNA from tobacco to the soil bacterium Acinetobacter sp. by natural transformation. Mol Microbiol. 2004;53:323-34. de Vries J, Wackernagel W. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proc Natl Acad Sci U S A. 2002, 99:2094-9

[42] Nielsen KM, van Elsas JD, Smalla K. Transformation of Acinetobacter sp. strain BD413(pFG4DeltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Appl Environ Microbiol. 2000, 66:1237-42.

[43] Nielsen KM, Bones AM, Smalla K, van Elsas JD. Horizontal gene transfer from transgenic plants to terrestrial bacteria--a rare event? FEMS Microbiol Rev. 1998, 22:79-103. Droge M, Puhler A, Selbitschka W. Horizontal gene transfer as a biosafety issue: a natural phenomenon of public concern. J Biotechnol. 1998, 64:75-90.

- 2/ the presence of resistance genes facilitates as "anchors" the integration into the bacteria of adjacent gene plants; these genes are expressed and can be disseminated via the bacteria in the environment. The efficiency of the gene transfer under natural conditions appears to be weak. However, this statement is based on a small number of experimental studies found in the literature.

C. The foreign DNA integrated into the genome of soil bacteria or non pathogenic bacteria has to be transferred to pathogenic bacteria

93. There are numerous evidences which show that DNA is easily exchanged through various ways between bacteria, including pathogens and non pathogens.

94. There may also be further considerations necessary to perform a thorough risk assessment of three ARMG, such as the presence, integrated into the plant DNA and genetically linked with the ARMG, of remaining important bacterial plasmid sequences and functions (origin of autonomous replication, horizontal mobility functions) of the vector initially used to transform the plant. These sequences may further confer autonomous replication of the ARMG if transferred to bacteria, or worst, wide host range transfer plasmid functions would enable the rapid spread of the ARMG to bacteria from other species or genera.

95. The general conclusion on the transfer of cloned ARMG from genetically modified plants to pathogenic bacteria is therefore that since each required step has been demonstrated as possible, a plausible scenario in which the cloned ARMG is transferred from genetically modified plants to pathogen bacteria can be built. This conclusion is supported by circumstantial evidence that indicates travelling of genes from plants to bacteria during evolution and confirms the likelihood of the scenario. However, in terms of risk analysis, the risk has not been properly quantified and is probably very low.

96. The next sub question is then, if ARMG from plants happen to be transferred to the bacteria, which risk for human health? Which risks for animal health? This relates to the antibiotics concerned by the ARMG from GM plants.

97. Since the risk will depend on the clinical and veterinary importance of antibiotics toward which resistance is conferred, a case-by-case analysis of antibiotics should be carried out. The major antibiotic resistance markers commonly used in GM plants are:

Kanamycin resistance gene: *npt*II gene

98. This gene is widely used as a marker. It confers resistance to kanamycin and neomycin by inactivation of the antibiotics. The use of kanamycin is marginal in therapy and resistance is of very weak clinical importance. However, amikacin is an important antibiotic for therapy in humans which is derived from kanamycin. Mutations in the *npt*II gene can extend the activity of the produced inactivating enzyme to amikacin. However, two mutations are required and have only be obtained under laboratory conditions and never detected so far in clinical isolates although there are clinical isolates which harbour the *npt*II gene.

Streptomycin resistance gene: *aad*A gene

99. The confers resistance to streptomycin and spectinomycin by inactivation of the antibiotics. The use of these antibiotics is marginal in therapy and resistance is of weak clinical importance. The only indications are multidrug resistant tuberculosis for streptomycin and uretritis due to gonococus

(gonorrhoea) for spectinomycin which are narrow but important indications. Streptomycin is used as a pesticide in agriculture, mostly in the USA and in Japan.

Ampicillin resistance gene: *bla*$_{(TEM)}$ gene

100. The gene confers resistance to ampicillin and related antibiotics by inactivation of the antibiotics due to an enzyme called penicilinase. Ampicillin is an important antibiotic for humans and animals. However, resistance to ampicillin is already widespread in pathogenic Gram-negative bacteria responsible for urinary tract and intra-abdominal (peritonitis) infections and this antibiotic is mostly used in respiratory tract infections mostly due to Gram-positive bacteria which do not produce beta-lactamases. 30%-50% of *E. coli*, the most common bacteria responsible for urinary tract infections, isolated from urines in Europe, the USA and Latin America produce penicillinases[44]. Most of the penicillinase-producing isolates contain a *bla*$_{(TEM)}$ gene.

101. These ARMG have also to be carefully considered as regards the relevant veterinary uses of the antibiotic concerned to treat animal diseases, in particular any epizootics or zoonosis.

102. Are all the antibiotics concerned by the resistance genes from GM plants of equal importance? As explained above, it appears that risk is different according to the usage of antibiotic. It is also different according to the previous spread of the resistance genes in nature. It appears that the *npt*II gene is already widely distributed among soil and enteric bacteria and that it confers resistance to non important antibiotics. The *aad*A gene is similar in importance; however, it is used in a very limited but important indications in therapy. The *bla* gene confers resistance to clinically important antibiotics but is already spread in nature.

103. Furthermore, there are other ARMG present in the GM products in dispute that may have different risk assessments, in the light of other important therapeutic uses of the corresponding antibiotics. This is for instance the case for the *nptIII* gene, conferring resistance to amikacin, as explained in our comments in relation to Q 98.

104. A scientific panel of the European Food Safety Authority[45] has evaluated the potential risks associated with specific antibiotic resistance genes and has recommended to classify the antibiotics in three risk groups, from the lowest to the highest: group 1 which contains antibiotic resistance genes already widely distributed among soil and enteric bacteria and confer resistance to antibiotics which have no or only minor therapeutic relevance, group 2 which contains antibiotic resistance genes already widely distributed in nature and confer resistance to antibiotics which are used for therapy in defined areas of medicine and group 3 which contains antibiotic resistance genes which confer resistance to antibiotics highly relevant for human therapy, irrespective of considerations about the realistic value of the threat. The group 1 contained the *nph*II gene, the group 2, the *bla* and the *aad*A genes and the group 3 other genes not discussed here. The panel recommended that the use of antibiotic resistance gene of group 2 should be restricted to field trial purposes whereas the panel found that there was no rationale for restricting the use of group 1 antibiotic resistance genes.

[44] Gordon KA, Jones RN; SENTRY Participant Groups (Europe, Latin America, North America). Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: comparison report to Europe and Latin America. Results from the SENTRY Antimicrobial Surveillance Program (2000). Diagn Microbiol Infect Dis. 2003, 45:295-301.

[45] Opinion of the Scientific Panel on Genetically Modified Organisms on the use of antibiotic resistance genes as marker ge,es in genetically modified plants. The EFSA Journal 2004:48;1-18. Available at <http://www.efsa.eu.int>

105. The notion that there were so far no reports in bacterial isolates from humans or animals that marker genes have transferred from GM plants to bacteria is not based on sufficient evidence: since the marker genes are the same as those already spread in nature, it is difficult to trace them.

106. This scientific evidence is the basis of the conclusions that have been outlined above by the European Communities.

Risk management considerations

107. Although the risk to human health from the transfer of antibiotic resistance to pathogenic bacteria is considered low, it is nonetheless significant, as the Codex Alimentarius has recommended that the presence of ARMG be avoided. The hazard from the gene transfer of ARMG clearly exists, and it is hard, if not currently impossible, to quantify precisely the corresponding risks by the current risk analysis methods. Accordingly, ARMG should be avoided.

108. This recommendation also takes into account the fact that these genes, which are required for the construction of the genetic modification, are no longer useful in the commercially available GM plants, and that techniques exist to remove the ARMG genes from the GM plants at the terminal steps of the construction.

109. This risk management conclusion is also justified by the fact that there is general policy in Europe, and to some extend more globally, to reduce the incidence of bacterial antibiotic resistance in pathogen bacteria. This is reflected in particular in the efforts that are made to monitor and control antibiotic consumption in humans and animals (campaigns to reduce consumption, controlled prescription in hospitals, ban of antibiotics used as growth promoters in animals) and recommendations for the approval of pro-biotics which should be devoid of "dangerous" resistance genes. These efforts should not be undermined by the use of ARMGs in GMOs.

Question2

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that antibiotic resistance develops through ways other than the uptake of food or feed by humans or animals, that is, due to the potential persistence of plant-derived DNA in the environment during crop cultivation and harvesting, and in soil residues?

(i) *If scientific evidence indicates that such an event could occur, what risks, if any, would arise from that event? What is the comparative relevance or magnitude of this risk in relation to the likelihood of such a transfer from other sources of antibiotic resistance not involving the use of recombinant DNA technology?*

(ii) *Are these consequences relevant to the specific types of ARMG currently used in the products at issue in this dispute?*

(iii) *If such risks have been identified, what risk management options are available to mitigate those risks and what is their efficacy?*

General comments

110. The Panel's second general question sought equally important advice as to whether other routes than food or feed, and persistence into the environment and in soil of plant DNA, may also lead to the development of antibiotic resistance.

111. Unfortunately no expert has responded to this important general question, Dr. Nutti stating that it is a question relating to environmental safety on which she does not have expertise. As it is a question intimately related to Question 1, the European Communities will endeavour to provide as objectively as possible scientific evidence to address the issue raised by the Panel.

112. From the scientific evidence presented below, it can be concluded that:

- antibiotic resistance may develop through ways other than uptake of food/feed by humans and animals and transfer to gut microorganisms;
- the demonstration has been obtained that resistance genes from GM plants can be captured and expressed by bacteria from the environment, probably at a low frequency;
- the risk for human or animal health (infection in immuno-compromised patients directly by the environment bacteria) may be low, but the hazard is significant.
- the same case-by-case analysis as in the comments on question 1 should be applied, as different ARMG, different genetic constructions, and different environments may bear significant differences in the associated risks.

Detailed comments

113. There is clear evidence that antibiotic resistance develops not only through the uptake of food but also through the circulation of bacteria in the environment and between humans and animals directly (by contact) or indirectly (by inert vectors). An example is the case of nosocomial infections (infections acquired in the hospital) which are mostly related to the acquisition by the patients of multiply resistant bacteria from other hospitalised patients or from insufficiently sterilized instruments. Another example is the acquisition of resistant bacteria through the consumption of water contaminated by bacteria from the environment, or from soil contamination of wounds for farmer or other workers exposed to soil. As regards to the question 2, this last example is the most relevant.

114. It must be remembered that we know only a minority of the bacterial and microbial flora in the soil. Probably up to more than 95 to 99% of the species are still unknown. Consequently, the consequences of a the presence of ARMG DNA in the soil, and its possible transfer to bacteria, cannot be fully assessed at this stage.

115. In soil bacteria, there are already a significant number of antibiotic resistant strains, including among pathogenic bacteria. On one hand, this would make that adding some more resistance genes, and probably in low frequency, has no heavy impact. But on the other hand, all efforts aiming at reducing the proportion of resistance genes in populations of bacteria, and in particular in unknown bacterial ecosystems, should be highly encouraged. Moreover, if it occurred that a pathogenic strain had become resistant as a result of the cultivation of GM plants, and that these bacteria caused fatal diseases, there would be no way to assess the origin of this event. There is no tracing of the resistance genes introduced in GMOs. From that point of view, adding ARMG genes in any part of the environment is certainly not acceptable.

116. On top of that, the genetic linkage in the GM plant of ARMG with other genetic elements might enhance the maintenance or amplification of ARMG DNA derived from GM plants in the environment. Indeed, these genetic elements linked to the ARMG, whether voluntarily present or not, such as bacterial plasmid origin of replication, bacterial plasmid origin of transfer and mobility functions, including wide host range transfer, or plant genes that would confer a positive selective advantage to that plant under specific conditions (e.g. an herbicide resistance gene in the presence of the herbicide) might confer selective advantages to the maintenance of the ARMG in the different cells and organisms where it might be integrated.

117. Furthermore, largely unexplored physico-chemical properties of the soil, such as its pH, may be very diverse and would have a very important impact on stability and transformability of naked DNA in the soil. In certain environmental and physico-chemical conditions, intact DNA has been showed to be able to persist for very long period of time in nature.

118. As mentioned in Question 1 above, we know that *Acinetobacter calcoaceticus,* an environmental bacteria, has been successfully transformed with plant DNA bearing an antibiotic resistance gene (kanamycin or streptomycin/spectinomycin resistance)[46]. The frequencies of transformation were relatively low. It should be noted that *Acinetobacter* is an important source of infections (particularly in fragilized and immuno-compromised patients) originating from the environment in hospitals and is considered as an emerging pathogen[47]. However, in hospitals, this bacteria is already resistant to a large panel of antibiotics and an additional resistance coming from plants would not significantly change the dramatic problem posed by multiple resistance of this bacteria.

119. This single example may however be the tip of the iceberg, that has gone uncovered because it was looked for, while other examples may also prove to occur and be more problematic in terms of therapeutic impact, but have not been discovered yet because they were not properly searched for. In addition the risk for spread of resistance in nature should be significant only if an antibiotic selective pressure was exerted by the use of antibiotics: the use of a selector antibiotic would amplify the number of bacteria with the resistance gene by eliminating the susceptible bacteria. This, nevertheless, does not exclude maintenance and movement of the ARGM in nature in the absence of the selection, or its amplification due to the selection of another genetic element linked to the ARMG.

120. Also, there is no absolute barrier between bacteria which could prevent gene transfer. Therefore, resistance could be transferred from plant to environmental bacteria and then to pathogenic bacteria in multi-step events. Again the probability of occurrence of this event should be very low, unless there is an advantage to maintain or transfer the ARMG.

121. Another important point is that, as already mentioned, the DNA segments integrated in *Acinetobacter* often encompasses plant genes which were adjacent to the ARMG gene, and that the bacteria expressed the plant gene[48]. The presence of ARMG genes facilitates as "anchors" the integration into the bacteria of adjacent plant genes; these genes can be expressed and can be disseminated via the bacteria in the environment. Again, in case of use of the selector antibiotic in agriculture, the presence of ARMG and any genetically linked gene might lead to a significant amplification of the number of bacteria and significant spread of the corresponding genes.

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[46] Gebhard F, Smalla K. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol. 1998, 64:1550-4. Nielsen KM, Bones AM, Smalla K, van Elsas JD. Horizontal gene transfer from transgenic plants to terrestrial bacteria--a rare event? FEMS Microbiol Rev. 1998, 22:79-103. Nielsen KM, van Elsas JD, Smalla K. Transformation of Acinetobacter sp. strain BD413(pFG4DeltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Appl Environ Microbiol. 2000, 66:1237-42. de Vries J, Herzfeld T, Wackernagel W. Transfer of plastid DNA from tobacco to the soil bacterium Acinetobacter sp. by natural transformation . Mol Microbiol. 2004;53:323-34. de Vries J, Wackernagel W. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proc Natl Acad Sci U S A. 2002, 99:2094-9.

[47] Coelho J, Woodford N, Turton J, Livermore DM. Multiresistant Acinetobacter in the UK: how big a threat? J Hosp Infect. 2004, 58:167-9. Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother. 2004,38:1449-59.

[48] de Vries J, Herzfeld T, Wackernagel W. Transfer of plastid DNA from tobacco to the soil bacterium Acinetobacter sp. by natural transformation . Mol Microbiol. 2004;53:323-34.

122. Here again, the risk management options proposed by the Codex as it relates to food safety, may be applied more generally, namely that the presence of ARMG should by all means be avoided, and should not be present in GM plants when the genes may potentially confer resistance to clinically and veterinary important antibiotics.

Question 3

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that wide-spread cultivation of Bt crops such as biotech maize of the Bt variety adversely affects non-target organisms which may be exposed to such crops under typical agricultural practice? (See, inter alia, EC-149, EC-150, EC-151, EC-152) If so, how does this risk compare with risks to non-target organisms arising from non-biotech applications for Bt toxins (i.e., the use of Bt toxin as an insecticide in conventional and organic farming)? What risk management options are available to mitigate any resulting risks and what is their efficacy?

General comments

123. The European Communities notes that the two of the Panel's experts who address this issue (Drs. Squire and Andow) agree that the effects of GMOs expressing the Bt toxin *may* have effects on non-target organisms, which they believe is not yet confirmed, as small scale releases show little immediate toxic or catastrophic effects. However the absence of observed catastrophic effects does not mean in the view of the experts no effects on non target organisms, and Dr. Andow advocates therefore that any risk management options should be based on worst case scenarios. Dr. Squire explains that the long term field scale monitoring will be necessary to determine whether there is such a significant effect. Such tests will be specific to the crop and the environment in which it is to be grown.

124. The European Communities has little detailed comment to add to this advice at the present stage, but would like to point to a recent body of scientific evidence of non target effects of Bt crops, in particular on soil organisms and nematodes, as well as a number of selected references relevant to non target organisms sensitivities to Bt toxins.

Detailed comments

125. As regards unintended effects of Bt maize on non target organisms, a very recent paper[49] shows some effects of the maize expressing the Bt toxin on soil nematodes, which are site and season specific effects, supporting the arguments for a) case by case studies b) long term monitoring (3 years minimum), c) region specific studies, d) and the inclusion of relevant comparisons (other crops and soil management treatments).

126. The effects detected on nematodes was also found by other authors[50], although no one yet understands why the effect happens (less nematodes under Bt maize in some soils/regions/tillage

[49] See Exhibit EC-164: B. S. Griffiths, S. Caul, J. Thompson, A. N. E. Birch, C. Scrimgeour, M. N. Andersen, J. Cortet, Antoine Messéan, Christophe Sausse, Bernard Lacroix, P. H. Krogh. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the Bacillus thuringiensis CryIAb toxin. Manuscript accepted in Plant and Soil Journal.

[50] B. Manachini, S., Landi, M. C. Fiore, M. Festa and S. Arpaia, IOBC wprs Bulletin, 2004, 27, 103. Manachini B., Lozzia G.C., 2002 - First investigations into the effects of Bt corn crop on Nematofauna. - Boll. Zool. Agr. Bachic. Ser. II, 34(1): 85-96. Manachini B., 2003 - Effects of transgenic corn on Lydella thompsoni Herting (Diptera:Tachinidae) parasitoid of Ostrinia nubilalis Hb. (Lepidoptera Crambidae).- Boll. Zool. agr. Bachic. Ser II, 35(2): 111-125.

regime combinations). It may be related to root exudation, plant composition, altered moisture below Bt maize plants, altered trophic interactions of a food source for nematodes, or any other effect directly or indirectly related to the expression of the Bt toxin. It strikingly shows evidence of continued scientific uncertainties and regional differences on soil ecology as regards non target effects of Bt maize. Some would say that the results are small and transitory, and could therefore be dismissed, but this is not an appropriate risk assessment end point if the effects accumulate over time due to repeated maize monoculture in the same field, due to the ability to grow some GM crops with no tillage and repeatedly over several seasons. In such circumstances, the risk to soil organisms and soil ecosystem could then increase over time.

127. The European Communities would further like to submit scientific evidence on differences in insect species/sensitivity to Bt toxins for a) pests, and b) non-pests (conservation or heritage species, like butterflies), which are both relevant for this question and Question 4.

128. The current state of Bt environmental risk assessment in Europe shows that there were and still are considerable grounds for concern about the toxin Bt, especially non-target effects, which have only been addressed in recent years and which still continue to produce large amount of data.

129. This additional scientific evidence on differential sensitivities to Bt toxins, available from peer-reviewed publications, show that between pest species and conservation or heritage species there is a wide range of sensitivities to Bt toxins expressed in GM crops. Hence it is possible to argue that:

– Scientific uncertainties about the **non-target species effects** of Bt toxins, on regional non-target insect sensitivities, still do exist (e.g. butterflies of regional cultural value that could be exposed to Bt toxins via the GM crop in any one region of Europe). (Bt toxin sensitivities for lepidopteran, non-pest species which could have regional- or country-specific conservation or heritage value. i.e. non-target herbivores (butterflies) that could be affected by Bt crops and show species differences in sensitivity to Bt toxins)[51]

– Even for **target pest lepidopteran species**, considerable differences in species sensitivities exist, which can impact on Insect Resistance Management (IRM) strategies for any particular EC country or region (Bt toxin sensitivities for lepidopteran (caterpillar) pest species which are potential 'target' species for Bt crops, showing Bt toxin sensitivity differences between species)[52]. i.e. A 'high dose + refuge strategy' for species X in one region may not be effective as a 'high dose + refuge strategy' for species Y in another region, because species Y is less sensitive to Bt toxin than species X. This effect has already been demonstrated[53] for Bt cotton grown in USA vs Australia, where the Bt cotton event produced in USA was reasonably effective in USA under the 'a high dose + refuge strategy' but failed to be sufficiently

[51] Wraight, Zangerl, Carroll & Berembaum (2001). Proc. Natl. Acad. Sci. USA 97: 7770-7773. Zangerl, McKenna, Wraight, Carroll, Ficarello, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 113-118. Sears, Hellmich, Stanley-Horn, Oberhauser, Pleasants, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 119317-42. Jesse & Obrycki (2000) Oecologia 125: 241-248. Jesse & Obrycki (2002). J. Kansas Entomol. Soc. 75: 55-58. Jesse & Obrycki (2003) Agric. Environ. 97: 225-233. Hellmich, siegfried, Sears, stanley-Horn, Daniels, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 11925-30.

[52] Fitt et al., 2004 Report for International Cotton Advisory Committee, pp 1-64. Felke, Lorenz & Langenbruch (2002). J. Appl. Entomol. 126: 320-325. Wolt, Peterson, Bystrak & Meade (2003). Environ. Entomol. 32(2) 237- 246. Dutton, Romeis & Bigler (2003). BioControl 27: 441-447. Bernal, Griset & Gillogly (2002). J. Entomol. Sci. 37: 27-40.

[53] Fitt et al., 2004 Report for International Cotton Advisory Committee, pp 1-64

effective against important Australian cotton pest species in the IRM because they were less sensitive to the Bt toxin.

130. Australia has now moved to a new Bt cotton event expressing two different Bt toxins to achieve a better 'high dose' strategy for Australian species which were less sensitive to first generation Bt cotton (especially at the end of the growing season).

131. This scientific evidence further points to regional differences and lack of sufficient information for both target (pests) and non target (biodiversity and conservation species).

Question 4

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that the wide-spread cultivation of Bt maize or other, non-biotech applications of Bt toxins, leads to the emergence of Bt-resistant target organisms under field conditions? If so, what risk management options exist to mitigate any resulting risks and what is their efficacy?

General comments

132. The European Communities notes that the two of the Panel's experts who address this issue (Drs. Squire and Andow) both agree that the development of Bt resistance in target insects is inevitable. They both agree that there are different risk mitigation techniques, and that there is still a large amount of uncertainties as regards the pace and impact of resistance development. According to Dr. Andow, the "high dose refuge" strategy is the most effective.

Detailed comments

133. The European Communities has nothing to add to this advice at the present stage, but would like to point to the further evidence it had submitted in relation to the previous question, namely as regards differences of Bt toxin sensitivity in different target organisms, that may warrant regional differences in risk management and integrated resistance management strategies (confer the differences that occur between the United States and Australia, that necessitates differences in mitigation approaches).

134. From an evolutionary and ecological point of view, it is clear, as pointed out by the experts, that adding the same genes in the genome of numerous crops, cultivated on vast neighbouring areas will, sooner or later, lead to the appearance of resistant organisms. How long it will take, is not known. A parallel may be drawn with dangerous uses that have developed in the past with antibiotics. The overstated confidence in our ability to find new molecules when the existing ones become inefficient has prevented a sustainable use of many chemicals. For example, antibiotics were a great discovery, among the greatest in medical sciences. If they had been properly used and managed, they would probably still be perfectly efficient. As soon as 1945, Flemming warned that unconscious use and abuse of these molecules would lead to the loss of their efficiency. This example holds for pesticides, and it is to be remembered that the exogenous Bt toxin is, in certain instances, the only technique currently available to some agricultural practices, such as organic farming.

Question 5

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that Bt maize varieties are any more toxic to humans or animals than conventional maize under field conditions? If so, what risk management options exist to mitigate any resulting risks and what is their efficiency?

General comments

135. In answering this question, Dr. Squire appropriately examines the evidence of harm (or of absence of harm) of growing Bt crops in Europe, and opines that the evidence of Bt maize to humans or animals is inconclusive at present, while acknowledging that the only sound indication that the current state of knowledge provides, is that there is no evidence of acute toxicity to mammals and most animals other than pests.

136. On the contrary, Dr. Nutti offers a characteristically short, negative, unsubstantiated answer, stating only that:

> Based on the information before the Panel, there is no evidence to support the hypothesis that Bt maize varieties are more toxic to humans or animals than conventional maize under field conditions.

137. Dr. Nutti's response is scientifically inadequate in terms of coverage and lack of any supporting evidence. There are numerous possible ways that Bt maize could be toxic (i.e. have an adverse effect on the receiving organism following acute or chronic exposure) when Bt maize is grown under European field conditions.

138. Because of the inadequacy, and incompleteness of the advice provided by Dr. Nutti to the Panel, the European Communities provides below its own analysis. The European Communities has prepared this information with the assistance of scientists, experts in the field of non target direct and indirect impacts, toxicity to organisms, and ecological assessment, and presents it as objectively as possible so as to complete the information available to the Panel. In so doing, the European Communities will interpret the term "animals" as including the wide range of non target organisms belonging to the animal kingdom (i.e., in addition to humans and domesticated mammals) which could be adversely affected by Bt maize, as would many international scientists researching non target effects of GMOs consider this term.

Detailed comments

139. It is not scientifically reasonable to simply translate and extrapolate the limited risk assessment results on the toxicity of Bt maize to human and non-target organisms from USA, Australia or some other non-European countries, because the

- regional growing environments,
- scales of farm fields,
- crop management practices,
- local/regional target and non-target species considered most important in the agro-ecosystem,
- interactions between cultivated crops, and
- surrounding biodiversity,

could each be different from published non-European studies and could differ substantially between regions and countries within the EC. Case-by-case risk assessment (including interactions between the GM crop and its local environment) is widely accepted as a valid approach for assessing possible environmental and health impacts of GM crops and foodstuffs.

140. Studies illustrating non-target effects and scientific uncertainties over non-target effects from the longer term exposure of Bt crops to appropriately selected non-target organisms, above and below ground, include the following:

141. Effects (either directly to the non-target organism, or indirectly to non-target organisms via simple or complex trophic interactions in the food web) of the Bt toxin itself, or of the Bt crop as a whole, on non-target insects which provide important 'ecological services' in agro-ecosystems.[54]

142. In several of the above non-target studies on Bt crops, the possibility is raised from a scientific viewpoint that sub-lethal effects detected in small scale or short term studies (e.g. lab or contained glasshouse) could possibly be exacerbated by longer term exposure (i.e. over a growing season encompassing multiple generations of non-target insect species) and by toxic interactions between expressed Bt toxins and other components of the non-target target organisms normal diet. For an herbivore, this intake of other toxic compounds which the Bt toxin could interact with neutrally, additively, synergistically or antagonistically, would include defensive plant chemicals produced constitutively or induced by plant herbivore damage.[55] These complex toxicological and behavioural interactions mean that simple risk assessment tests on non-target organisms without using the growing GM crop in a realistic and appropriate environment are not conclusive to indicate a meaningful degree of risk (or not).

143. Several examples (cited in the studies referenced previously) of unintended or unanticipated effects arising from transformation of transgenic crops, show that plant metabolites involved in ecological interactions with non-target organisms can be changed during production of Bt crops, and have the potential to influence the toxicity of Bt toxins by unpredictable toxicological interactions as part of the complex diet consumed by herbivores.

144. From many ecological and eco-toxicological studies[56] it is clear that plant toxins and other plant defensive chemicals can in general have markedly different effects on the receiving organism, depending on the quantity of both toxin dose and exposure time (termed the 'hormesis' effect). Thus, quantitative changes (increases or decreases) in expressed Bt toxins and/or in plant metabolites affected by the transformation process have the potential to influence the overall 'toxicity' or environmental impact of the Bt plant for the herbivore and other non-target organisms at higher trophic levels in a local agro-ecosystem (meaning the Bt crop and the non-cultivated vegetation associated with the local cropping system of the Bt crop).

145. Scientific uncertainty is increased due to the ongoing scientific debate over the most appropriate testing methods and scales (spatially and temporally) useful to determine realistic ecological effects of Bt crops.[57] According to Levidow's review[58], a precautionary approach identifies

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[54] References: Hilbeck et al., 1998, Environ. Entomol. 27: 480-487. Hilbeck et al., 1999, Ent. Expt. Appl. Et Appl 91: 305-316. Saxena, Flores and Stotsky 1999; Nature 402: 480. Lovei 2001; Proc. 54th conf NZ plant prot Soc 54: 93-100. Bernal et al 2002; Ent Expl et Appl 102: 21-28. Groot & Dicke 2002; Plant Journal 31(4): 387-406. Oberhauser & Rivers, 2003; Agbionet 5: 1-7. Zwahlen et al., 2003; Mol Ecol 12: 765-775. Knols and Dicke 2003; Nature Biotech 21(9): 973-974. Fitt 2003; Proc. 3rd World Cotton Research Conf, Cape Town, pp 371-381. Conner et al 2003; Plant Journal 33: 19-46. Poppy & Sutherland 2004; Physiol Ent 29: 257-268. Wilson et al 2004; Technical report for EcoNexus. Andow and Hilbeck 2004; BioScience 54: 637-649. Lovei and Arpaia 2005; Ent Expt et Appl. In press. Andow and Zwahlen 2005 Ecology Letters In press.

[55] Birch et al 2001; Ann Appl Biol 140: 143-149. Haslberger 2003 Nature Biotechnology 21 (7) 739-741.

[56] See for a review and summary, Calabrese and Baldwin 2003 Nature 421:691-692.

[57] Levidow 2003 J Invert Path 83: 113-117. Dutton et al., 2003; BioControl 48: 611-636. Andow and Hilbeck 2004; BioScience 54: 637-649. Poppy & Sutherland 2004; Physiol Ent 29: 257-268.

new unknowns, and scientific questions, generates different criteria for evidence, and make judgements on environmental risk assessment more transparent. In his review, this is illustrated by the questions raised by the use of "surrogate" Bt toxin (i.e. a toxin artificially isolated from an heterologous expression systems which is different from the Bt maize considered), in order to test the toxicity of the isolated Bt toxins on non target organisms. In reality, the non target herbivore will consume the Bt toxin produced by the homologous Bt-expressing plant, which may therefore be different in molecular structure, size, posttranslational modifications, and biological activity to the artificially produced "surrogate" Bt toxin[59].

146. Bt toxin exposure routes for non target organisms, like insect pollinators, predators and parasitoids, are frequently multi-trophic[60], *not* simply bi-trophic that is, they arise from indirect complex and network interactions. Simplified bi-trophic testing systems, based on eco-toxicological models (as featured in many non target methods using a purified Bt toxin in artificial diets for non target insects) are now not considered by many experts to be ecologically realistic for assessing multi-trophic interactions of Bt crops over several insect generations and spanning at least three trophic levels (see eg following references [61]).

147. The views summarised in these cited publications focus on the need for multi-trophic testing, using real Bt crops in realistic testing conditions, tested with regionally appropriate non-target species, likely to be exposed to Bt toxin or metabolites. These views arise from accumulating scientific knowledge over the period from the mid 1990s until today. Scientific uncertainties on effects of Bt crops on non-target species or ecological functions were (and still are) identified and are being addressed by many international scientists.

148. Other scientific uncertainties arise from incomplete knowledge of the transgene locus structure and expression, of different sized Bt toxins, which can interact in uncertain ways in different environments. GM crops should be tested under regionally representative environments and appropriate crop management practices,[62] and without precise molecular characterisation data, it is not possible to be certain about correct doses and exposure routes for non-target organisms in the local agro-ecosystem.

149. Often the measured 'effect' (positive, negative, or neutral) of the GM crop on selected non-target species or ecological functions (e.g. predation, parasitism, pollination) is compared with one or more other treatments in the experiment (e.g. an isogenic line with/without chosen pesticides, or local varieties +/- application of chosen pesticides, or a local crop variety grown under Integrated Pest Management or under organic production, etc). This wide range of comparators, as 'baselines', to determine any change caused by growing the GM crop, from 'norm' equating to the selected baseline, means it is very difficult or impossible to generalise or extrapolate between studies, even within one country and for one GM crop event. This enforces the scientific arguments for regionally specific, case-by-case studies of GM crop impacts performed over several (preferably 3 or more) growing seasons in the same region.

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[58] Levidow 2003 J Invert Path 83: 113-117

[59] Levidow 2003 J Invert Path 83: 113-117. Freese and Schubert 2004 Biotechnology and Genetic Engineering 21: 299-324.

[60] Groot & Dicke 2002; Plant Journal 31(4): 387-406.

[61] Birch et al. 1998, 199. Hilbeck et al., 1998, Environ. Entomol. 27: 480-487. Hilbeck et al., 1999, Ent. Expt. Appl. Et Appl 91: 305-316. Groot & Dicke 2002; Plant Journal 31(4): 387-406.Oberhauser & Rivers, 2003; Agbionet 5: 1-7.Capalbo et al., 2003; J Invert Path 83: 104-106. Andow and Hilbeck 2004; BioScience 54: 637-649. Birch et al., 2004 In: Environmental risk assessment of Genetically Modified Organisms Vol 1: a case study of Bt maize in Kenya. Ch4,. Eds Hilbeck and Andow, CABI, UK, pp117-187

[62] Andow and Hilbeck 2004; BioScience 54: 637-649. Freese and Schubert 2004 Biotechnology and Genetic Engineering 21: 299-324. Wilson et al 2004; Technical report for EcoNexus.

150. Question 5 presupposes suitable access to scientific knowledge applicable under 'field conditions'. Scale of testing has a major impact[63] on environmental impact assessment (e.g. toxicity of Bt crops to non-target organisms). Scale-dependent effects of Bt crops can thus only be determined by studying their impacts over a wide range of spatial (labs, glasshouses, fields, landscapes) and temporal (hours, days, seasons) scales, under the localised environmental conditions (regions within countries) and using crop management systems regionally appropriate to were the Bt crop event is proposed to be grown. Generation of data to see the 'bigger scientific picture' generally takes several years of detailed research in several countries (see for instance the EU ECOGEN project as an example of suitable testing scales over multiple regions, countries and seasons, with Bt maize management practices compared, e.g. conventional versus minimum tillage). This project will only produce final reports in 2005/6 after 3-4 years of GM crop field trials in Denmark and France.

151. The European Communities would also like to address the comparison of Bt maize toxicity with conventional maize under field conditions. As must now be self evident to the Panel, biology is a complex and empirical science. As such, it allows to predict situations when they have been repeatedly tested (in order to make the necessary statistics). "Natural", sexual, crosses give results which we cannot predict, but we have evidence that, statistically, they produce harmless genotypes. They are not harmless because they are natural, but because they are familiar.

152. On the contrary, a GM crop, where a new Bt gene is introduced into its genome, leads to lots of unpredictable interactions. One of the important discoveries of genomics is that complex organisms, such as a human beings, can be produced by a number of genes which is not more than that of a plant such as maize. One of the conclusions from this understanding is that natural gene interactions are numerous and important, that they form complex webs of gene regulations, and that these interactions statistically reach genome-wide equilibrium in "conventional" organisms. No one can scientifically claim to be able to predict all consequences of the presence and functioning of a new gene (and even less for several) in a genome which has never been exposed or contained this gene. The potential hazard here is not a consequence of the action of modification the plant genome, but of the fact that it generates high levels of unpredictability. The risk here may not come from the genetic modification itself, but from the extreme unpredictability of the direct and indirect effects of the introduction of a new gene(s) and gene product(s) into the plant genome and its gene expression. This cannot be compared to conventional maize, where such new combinations have ever occurred.

153. In the face of this unpredictability, one possible way forward is the implementation of thorough risk management and monitoring programs. However, this leads also to very significant methodological problems, as it is first necessary to collect extensive "baseline" data, before a monitoring plan can be meaningfully implemented for such complex effects. Without appropriate control data (namely the state of the ecosystem and the environment prior to the introduction of the Bt maize), which is very difficult, expensive, tedious and time consuming to collect, monitoring plans and general surveillance may not be able to detect and identify significant unintended effects, or long term, sub-chronic toxicity on non target animals.

Question 6

On the basis of the information before the Panel, is there any scientific evidence to suggest that herbicide tolerant crops (whether biotech or developed through mutagenesis) are more persistent in the agricultural environment or more persistent in the non-agricultural environment than their conventional counterparts? If so, do herbicide tolerant crops qualify as a potential "pest" as the term is used in International Standard for Phytosanitary Measures (ISPM) 11?

[63] Joao 2002 Environmental Impact Assessment Review 22: 289-310

(i) *What is the potential for the establishment and spread of herbicide tolerant plants arising from handling, spillage during transport of the plant/plant parts, or any other means outside of cultivation in the absence of application of the herbicide? How is any potential for establishment and spread affected by environmental conditions, the presences of wild or conventional relatives of the herbicide tolerant plants in an area, or other factors?*

(ii) *What is the potential for the establishment and spread of herbicide tolerant plants in the presence of herbicide application (in fields, urban, domestic or other environments)? How is any potential affected by the existence of feral related plant species; infertile wild relatives; seed survival in relevant pedoclimatic conditions; the reproduction biology of the species; or other factors?*

(iii) *Is this potential different for biotech crops tolerant to two wide-spectrum herbicides? Please explain.*

(iv) *If significant risks of establishment and spread have been identified, what risk management options exist to mitigate any resulting risks and what is their efficacy?*

(v) *What types of post-market monitoring and data collection activities could be envisaged on the basis of the monitoring and review principles described in ISPM 11?*

General comments

154. Drs. Snow and Squire have provided long and useful answers to the question and all sub-questions, with a significant amount of detailed evidence to substantiate their respective opinions. Dr. Andow has also provided a useful, substantiated answer, although he only addresses the theoretical framework of the main question, and the last sub-question on post market monitoring. Together, even if they do not always agree among themselves, all three experts have provided convincing evidence in the field of the potential difficulties that are linked with herbicide tolerant (HT) crops. Their replies provide in particular useful evidence as regards the issues arising from the combined use of the HT crop and the corresponding herbicides, or from the combination of several HT traits, and in particular in the context of glyphosate and/or glufosinate HT, for which HT crops can only be currently obtained by way of genetic modification.

155. Some of the points made by the experts that the European Communities would like to note are: all three experts indicate that the available evidence demonstrates persistence of HT crops in the presence of herbicides, and Dr. Snow points also to the available evidence of the emerging problems with HT weeds. She also points to the difficulties to eliminate the current problems of spread of some glyphosate or multiple resistant HT crops in Canada. Dr. Squire identifies the existence of risk management options, but stresses the main difficulties arising with oilseed rape in Europe. He also indicates that monitoring schemes do not yet exist, although research is in progress, and that he is not certain whether it would be feasible to monitor events with low frequency occurrences. Dr. Andow indicates also that any ecological effects would be scale dependent.

156. By way of contrast, Dr. Nutti has again provided a short and unsubstantiated answer, which contradicts the three other experts, and addresses only the "pest" qualification. She only states, after recalling the pest definition in ISPM 11:

> In my opinion, herbicide tolerant crops can not be qualified as "pest", according to this definition.

157. Dr. Nutti has probably misunderstood the question as asking her to provide a judgement on the overall risk of GM HT crops on plant or plant products, while other experts have answered the question as to whether they could *potentially qualify* as a pest. Dr. Squire states that:

> Oilseed rape and beet are both crops and pests, as are many other crop plants (pests as defined in ISPM-11, page 6).

158. Dr. Snow also states that, while each type of herbicide-tolerant crop should be considered individually and that it is not possible to generalize across all herbicide-tolerant crops,:

> A crop with herbicide tolerance can qualify as a potential "pest" under the ISPM 11 definition if 1) it transmits herbicide resistance to weedy volunteer plants or the crop's sexually-compatible wild relatives, and 2) this leads to worse weed problems. Volunteer oilseed rape and its sexually-compatible relative, Brassica rapa, are considered to be weeds because they compete with food crops and require management.

159. Although Dr. Snow does not address in this statement the potential weediness and pest problems of volunteers in crop rotation, the European Communities agrees with these experts that GM HT crops do qualify as pests as defined in ISPM11.

160. The European Communities notes Dr. Andow's remark that:

> Thus I conclude that GM HT crops can qualify as a potential "pest" as the term is used in the International Plant Convention's (IPPC) International Standard for Phytosanitary Measures (ISPM) 11 (EC-130). However, not all of the risks associated with GM HT crops can be considered phytosanitary risks.

161. Dr. Andow is correct in noting that not all of the risks associated with GM HT crops can be considered phytosanitary risks. Some are environmental or other risks rather than phytosanitary risks.

162. Considering the complexity of the question and sub-questions of the Panel, the importance of the issues raised for the European Communities, and the disagreement between the experts, the European Communities provides below its own analysis. The European Communities has prepared this information with the assistance of scientists, experts in the field of agricultural management and agro-ecological impacts of HT crops. The European Communities may address some sub-elements of the replies separately, where necessary.

Detailed comments

163. As a first general comment, the European Communities would like to clarify the following: as indicated by the experts, a plant with an herbicide resistance will easily become a pest if the herbicide is widely used. The consequence is that the same HT should not be introduced in different species that may be used in the same agricultural system, if no potential adverse consequences are to be expected. This is obvious, and is a systemic risk rather than a case-by-case problem. This is the issue that recently came to light when HT wheat was considered for approval in Canada and the United States, while several other crops had already been cultivated with the same GM HT character, including

oilseed rape. Furthermore, herbicides are not only used to remove weeds or volunteers in the fields but also on road verges, railways, gardens, industrial plants, etc.

164. Some species, like rapeseed, are already very aggressive outside and inside cultivated fields. As discussed by the experts, introducing resistance to important herbicides in certain species may certainly lead to significant spread and impacts. A problematic (worst) strategy would then be to introduce HT to several of the same relevant herbicides in one plant species with weediness potential. Cross hybridization would rapidly lead to the existence of multi-resistant plants which would then become major pests. As discussed by Dr. Snow, the fast appearance of such plants has already been demonstrated in Canada, and proves extremely difficult to manage.

165. Concerning sugar beets, their easy hybridization with wild relatives give birth to very invasive weeds in Europe. Introducing resistances to herbicides in cultivated beets would also lead to significant agro-environmental impacts, as it would also rapidly lead to HT weeds, probably within a couple of years.

166. A weed is an unwanted plant in the field that competes successfully with the crop plant for resources and thereby interferes with our goal of maximizing yields. A weed becomes more noxious the better it can escape measures of control by the farmer. There are various strategies, by which such noxious plants can do that. One is to mimic the crop plant and thereby being indistinguishable for the farmer from the crop during the time period it could be controlled (typically early season). Thus, many crop plants have close relatives that became tough weeds/competitors of them. Examples are: rice/weedy rice, maize/teosinte, oilseed rape and several oilseed rape-relatives, sugar beet/beta maritima etc.

167. The major means for control in industrialized, large-scale farming is by using chemicals, i.e. herbicides. Therefore, another very effective strategy for competitive weeds is to develop resistance against herbicides. The more intensive the control measure the larger the selection pressure to develop resistance and the quicker it develops. Weeds have and still do exhibit an astounding capacity of developing resistances against almost all compounds ever used against them and this happens in a way highly correlated to the amount and types of herbicides used against them (One of the most authoritative sources of information on the current status of resistant weeds worldwide is the "International Survey of Herbicide Resistant Weeds" that can be found at http://www.weedscience.org).

168. However, until recently, the plants had to develop these resistance mechanisms at least by themselves. Since the introduction of HT crop plants, we actually put HT-resistance genes actively out into the agro-ecosystem and make them available to any crop relative. All it takes ever since for related weeds, notably often bad competitors already, is to take these offered HT genes up, they do not even have to develop them anymore. This shortcuts the resistance development process of related and unrelated weeds significantly. In addition, we increase the selection pressure tremendously by exclusively spraying the complementary herbicide.

169. As already indicated, HT crops are only more persistent in environments where the specific herbicide or a closely related chemical with similar physiological activity is used. There is no indication that they are more fit or competitive in other environments.[64] Indeed the continuous production of enzymes to degrade or inactivate the herbicides could reduce resources available to the crop/plant. Studies of isogenic lines with and without GM HT suggest no difference in crop

[64] Crawley et al. 2001.

performance and thus no differences in ecological fitness.[65] But then of course the maintenance in the agro-ecosystem and the seed bank of the HT character leaves it available for any situation in the future where the selection pressure will again be applied.

170. The persistence of the crop is case specific and depends largely on the crop species and variety. In Europe the oilseed rape grown is predominantly winter type (which requires vernalisation) and persists much more in milder European conditions. There is no winter kill and high persistance of seed banks in soil. This is a different crop from North American spring oilseed rape which does not survive winters except in seed banks and is not so invasive.

171. It also depends on the regions in Europe where crops are grown. For example maize survives very poorly in regions with colder winters. But in warm maritime regions maize seeds and volunteer seedlings will persist and survive during winter and so could occur in subsequent crops or their field margins.

172. If so, do herbicide tolerant crops qualify as a potential "pest" as the term is used in International Standard for Phytosanitary Measures (ISPM) 11 ?

173. With HT crops there is two different risks: a) the HT crop becomes itself a weed in situations where the respective broad spectrum herbicide is used, b) the HT gene is conferred to related weed species exacerbating their weediness by the acquired new resistance.

174. All crops can be weeds (pests) when they grow as volunteers in subsequent crops and compete with them. Different crop species vary in their competitiveness and ease of control. Often they are controlled by the herbicide programmes used to control weeds. However specific treatments may be required to remove some of them. HT crops (especially oilseed rape) have become problematic when they have occurred adventitiously in other crops without farmers realising it and having tried to use the specific herbicide to control them without success. This means that they have suffered losses of yield or quality when they normally would not. In some cases different GMHT oilseed rape types have hybridised resulting in volunteers with multiple herbicide tolerance and can be more difficult to manage[66].

175. Adventitious presence is particularly a potential problem in Europe where GM oilseed rape seeds shed at harvest may persist in soil for several years and emerge in subsequent non-GM rape fields. These GM plants will then cross pollinate with conventional rape to the extent that thresholds for presence of GM seeds in conventional crops may be exceeded[67].

176. Glyphosate is widely used in agriculture today in combination with sterile seed bed techniques (i.e. cultivation of ground to encourage weed germination and then spraying with glyphosate to remove the weeds) as a means of reducing weed pressure on farms. In addition glyphosate and glufosinate are used as dessicants of crops with the added advantage that they kill weeds maturing in the crops just before harvest and reducing weed seed return to soil. Growing glufosinate and glyphosate tolerant crops would restrict farmer's ability to conduct these practices and

[65] SIMPSON, E & SWEET J B (2002). Consequence analysis of herbicide tolerant oilseed rape. Report for DEFRA, Project RG 0217., 55pp.

[66] ORSON J (2002): Gene stacking in oilseed rape: lessons from the North American Experience. Report for English Nature 2002: 17pp.

[67] SWEET, J. B., SIMPSON E. LAW., J, LUTMAN, P.J., BERRY, K., PAYNE, R., CHAMPION, G., MAY, M. WALKER, K., WIGHTMAN, P., & LAINSBURY, M. (2004). Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT Project). HGCA Project Report No 353, 265 pp. (www.hgca.com)

they would have to find other methods for managing weeds on their farms, which may entail extra costs or use of herbicides with a worse environmental profile.

177. However, Sweet et al (2004) also concluded that HT volunteers of rape and beet could be potentially controlled in non-related crops in rotation with appropriate management, assuming farmers were fully aware of their presence and the potential problems, and were prepared to accept the additional management load and possible economic consequences. This may however prove to not always be the case.

(a) First sub-question

> *(a) What is the potential for the establishment and spread of herbicide tolerant plants arising from handling, spillage during transport of the plant/plant parts, or any other means outside of cultivation in the absence of application of the herbicide? How is any potential for establishment and spread affected by environmental conditions, the presences of wild or conventional relatives of the herbicide tolerant plants in an area, or other factors?*

178. The European Communities believe that this question warrants an assessment highly specific to the case at hand. Dr. Squire states that:

> In the absence of the specific herbicide to which the plants are tolerant plants having HT and non-HT traits should act similarly.

179. But he further points out that:

> Species would differ (…) and spread depends on local conditions in ways that are not clear.

which justifies a thorough in-depth analysis of each considered crop.

180. As indicated above, the potential for establishment and spread is the same as for conventional crops and will only increase in the presence of the herbicide. The potential for establishment will depend on the crop species and the environment. The crops with the greatest potential are crops like oilseed rape which can readily colonised disturbed ground, e.g. building sites, road verges etc.

181. One should consider that some crops have a high potential for dispersal and long term establishment, especially crops like oilseed rape, which can be influenced by a range of environmental and agronomic factors. GM oilseed rape will behave in the same way. In addition crops like oilseed rape and beet have wild relatives with which they can hybridise.[68] Thus the presence of these related species will result in hybrid formation and introgression of genes into these wild species. The wild

[68] BARTSCH, D, CUGUEN, J, BIANCARDI, E, and SWEET, J (2003) Environmental implications of gene flow from sugar beet to wild beet – current status and future research needs. Environ. Biosafety Res. 2, 123-127 . Anne-Marie Chèvre, Henriette Ammitzbøll, Broder Breckling, Antje Dietz Pfeilstetter, Frédérique Eber, Agnès Fargue, César Gomez-Campo, Eric Jenczewski, Rikke Jørgensen, Claire Lavigne, Matthias S. Meier, Hans C.M. den Nijs, Kathrin Pascher, Ginette Séguin-Swartz, Jeremy Sweet, C. Neal Stewart Jr. & Suzanne Warwick (2004). A review on interspecific gene flow from oilseed rape to wild relatives. In: Hans C.M. den Nijs, Detlef Bartsch & Jeremy Sweet (Editors 2004) Introgression from genetically modified plants into wild relatives and its consequences. Proceedings of an ESF AIGM conference, Amsterdam, 21-24 January, 2003, 235-252.

population can then act as a potential reservoir over space and time for transgenes[69] or can become a weed in HT crops.[70]

182. Crop specific cases are discussed below:

Case of rapeseed

183. A retrospective study has been carried since 1996 in Selommes (Loir et Cher, France). The aim was to determine the origin of feral populations in road and field borders around a silo[71] and the impact of border management practices on their persistence.[72] In summary, this study demonstrates that seeds do disperse a lot. A field of rapeseed produces about 75000 seeds per square meter. About 10% are lost during harvest and transportation. It has been shown that these seeds establish all around the fields and roads where they are transported and that the plants could live and reproduce outside of the fields. It was demonstrated that some plants found outside the fields corresponded to genotypes which had not been cultivated for more than 10 years.

184. Several hypothesis concerning the origins of these plants were raised: spillage during sowing or harvest of adjacent fields, losses during transport,[73] "auto recruitment". A great diversity of situations and practices was observed,[74] depending on farming systems and landscape patterns. A complementary study determining the fitness of these populations was also carried out (Deville *et al*, 2002; 2003). Although these populations seems to have a lower fitness than those issued from the fields (Deville, 2003b), they produce seeds and persist in the environment.

185. Indeed, Pessel *et al* (2001) showed evidence that seeds could persist 8 years in road verges near cultivated area. Single-low rapeseed plants (low erucic/high glucosinolates) were still observed eight years after corresponding varieties had been withdrawn from the market. Lutman *et al*.[75] observed persistence of buried seeds of rape over time. They concluded in 2003 that "*Appreciable*

[69] EASTHAM, K & SWEET, J B (2001) Genetically Modified Organisms: the significance of gene flow through pollen transfer. European Environment Agency, Environmental Issue Report 28, 75 pp.

[70] Norris, Carol, Jeremy Sweet, John Parker & John Law (2004) Implications for hybridization and introgression between oilseed rape (Brassica napus) and wild turnip (B. rapa) from an agricultural perspective. In: Hans C.M. den Nijs, Detlef Bartsch & Jeremy Sweet (Editors 2004) Introgression from genetically modified plants into wild relatives and its consequences. Proceedings of an ESF AIGM conference, Amsterdam, 21-24 January, 2003,107-124

[71] Pessel, F. D., J. Lecomte, V. Emeriau, M. Krouti, A. Messean, and P. H. Gouyon. (2001). Persistence of oilseed rape (Brassica napus L.) outside of cultivated fields. Theoretical and Applied Genetics 102:841-846.

[72] Deville A. Garnier A., Lecomte J., Adamczyk K. Huet S., Merrien A., Messéan A., 2003a. Origin and dynamics of feral oilseed rape populations. Proceedings of the First European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Borupsgaard, 13-14 November 2003, pp 100-102. The Fate of Feral Plants; Jane LECOMTE In Commission du Génie Biomoléculaire, 2003. Impact sur l'environnement des cultures de colza génétiquement modifié tolérant à un herbicide. Actes du séminaire du 28 novembre 2003, 80p.; English version in press.

[73] Pessel, F. D., J. Lecomte, V. Emeriau, M. Krouti, A. Messean, and P. H. Gouyon. (2001). Persistence of oilseed rape (Brassica napus L.) outside of cultivated fields. Theoretical and Applied Genetics 102:841-846. Crawley M.J., Brown S.L., 1995. Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proc. of the Royal Society of London, B 259:49-54.

[74] Deville A. Monod H., Lecomte J., 2003b. Are rapeseed feral populations adapted to their environment? Proceedings of the First European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Borupsgaard, 13-14 November 2003, p 208.

[75] Lutman P. J. W.,Freeman S. E. Pekrun C., 2003. The long-term persistence of seeds of oilseed rape (Brassica napus) in arable fields The Journal of Agricultural Science, 141:231-240

numbers of rape seeds will persist up to 4 years, in normal cropping conditions and in the absence of cultivation, one experiment has confirmed persistence for over 11 years".

186. These results are consistent with those obtained in the BRIGHT study[76]. Gene flow between feral populations and fields was demonstrated by Devaux *et al* (2003).[77] Therefore, rapeseed and consequently HT oilseed rape plants has a potential to spread and persist over time in non-cultivated areas. Crop management practices in cultivated fields as well as road verges management have a great influence on survival and development of such feral populations (see below).

Case of weed beets

187. In Europe, four forms of the species *Beta vulgaris* exist:

- Wild or ruderal beet (*B. vulgaris* ssp. *maritima*)
- Sea beet (*B. vulgaris* ssp. *maritime* and ssp. *adanensis* in the eastern Mediterranean area)
- Cultivated beet (*B. vulgaris* ssp *vulgaris*)
- Weed beet (*B. vulgaris* ssp. *maritima*).

188. These forms of beet are interfertile, as shown by the gene flow detected between wild and cultivated beets[78] and by the transfer of useful genes of ssp *maritima* to new varieties of sugar beet.[79]

189. Forms of *Beta vulgaris* L. were first recognised as weeds in the 1950's,[80] but this problem was not really taken seriously in many European countries and the USA until the 1970's, when a large number of studies were initiated.[81] The presence of weed beet in sugar beet crops results in decreases in sugar yield (approximately 10% sugar yield loss per weed beet plant per m²) [82] and difficulties with harvest and sugar extraction. These problems result from differences in the reproductive cycle, as sugar beet is biennial and weed beets are annuals. The management of weed beet infestations has varied greatly and resulted in a large range of weed beet densities. No traditional chemical treatment

[76] SWEET, J. B., SIMPSON E. LAW., J, LUTMAN, P.J., BERRY, K., PAYNE, R., CHAMPION, G., MAY, M. WALKER, K., WIGHTMAN, P., & LAINSBURY, M. (2004). Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT Project). HGCA Project Report No 353, 265 pp. Available at <www.hgca.com>.

[77] Devaux C., Klein E. K., Lavigne C. 2003. A first step for modelling pollen dispersal at landscape level: determining the shape of dispersal functions at long-distance. The case of oilseed rape. Proceedings of the First European Conference on the Co-existence of Genetically Modified Crops with Conventional and Organic Crops, Borupsgaard, 13-14 November 2003, pp 172-174.

[78] Santoni S. and Bervillé A., 1992: Evidence for gene exchanges between sugar beet (Beta vulgaris L.) and wild beets: consequences for transgenic sugar beets. Plant Molecular Biology 20: 578-580. Boudry P., Mörchen M., Saumitou-Laprade P., Vernet Ph., Van Dijk H., 1993. The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. Theoretical and Applied Genetics, 87: 471-478. Bartsch D. and Schmidt M., 1997. Influence of sugar beet breeding on populations of Beta vulgaris ssp. maritima in Italy. Journal of Vegetation Science, 8: 81-84. Arnaud J.F., Viard F., Delescluse M., Cuguen J., 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in Beta vulgaris (Chenopodiaceae): consequences for the release of genetically modified crops species with weedy lineages. Proc. Roy. Soc., London, B270, 1565-1571.

[79] Debock T.S.M., 1986. The genus Beta: domestication, taxonomy and interspecific hybridization for plant breeding. Acta Horticulturae 182: 335-343

[80] Longden P.C., 1980. Weed beet. Agricultural progress 55: 17-25.

[81] Hornsey K.G. and Arnold M.H., 1979. The origins of weed beet. Annals of Applied Biology 92: 279-285

[82] Longden P.C., 1989. Effects of increasing weed-beet density on sugar-beet yield and quality. Annals of Applied Biology 114: 527-532.

can eliminate weed beets as they belong to the same species as sugar beet and are resistant to the same herbicides. In France, 4.5% of the sugar beet cropping area (20,000 ha) is infested with weed beet to such an extent that sugar beet crop production has had to cease because yields are too low. Weed beet has been reported in crops other than sugar beet since 1974.[83] Weed beet plants have never been considered troublesome as a weed in cereal crops because they are not very competitive and are easily controlled by herbicides.[84] They have also been identified as a common weed in pea (*Pisum sativum* L.) and potato (*Solanum tuberosum* L.) crops.[85]

190. The origin of weed beet was determined in 1993 from genetic studies[86]: accidental hybridisation seems to have occurred in the seed production area between a plant with cytoplasmic male sterility and a wild pollen donor from the *maritima* subspecies. Whereas most cultivated forms of beet are biennial, wild beets are mostly annual (dominant B allele). Hybrids between the two forms contaminating seed lots are sown at the same time as the seeds of the cultivated variety and grow in the row. It becomes possible to detect them when they begin to bolt and to flower. Weed beets may also originate in two other ways[87]: 1) Possible bolting of cultivated sugar beet due to low temperatures in the spring, vernalising the most sensitive plants so that they bolt and flower instead of producing reserves of sucrose; 2) Potential re-growth of the roots and neck, which remain on the ground after harvest. The plants resulting from such re-growth are in their second year of development and they bolt and produce flowers and seeds in the crop after sugar beet if they are not managed. These flowering beets then produce seeds, thereby maintaining the seed bank in the soil. Weed beet seeds have been demonstrated to survive in soil for considerable periods of time and it is therefore imperative for growers to prevent seed set.

Case of maize

191. Grains dispersed during harvesting, which could theoretically germinate, flower and pollinate the next maize crop, do not generally survive for more than a year. Maize is susceptible to cold and frost, and climatic conditions in Europe minimise its chances of long-term survival. Indeed, in south-west France, where maize is frequently grown in continuous cropping systems and where winter temperatures are mild, the maximum number of volunteers observed is 100 plants per ha. This number might be higher in southern countries like Spain where surveys are being carried out. This level of infestation would be sufficient to cause 0.9% contamination, but the volunteers are easy to pick out and to kill because they are located outside of the sowing lines in the field.

(b) Second sub-question

> *(b) What is the potential for the establishment and spread of herbicide tolerant plants in the presence of herbicide application (in fields, urban, domestic or other environments)? How is any potential affected by the existence of feral related plant*

[83] Gunn J.S., 1979. Weed beet in other crops of the arable rotation. British Sugar Beet Review, 7-10.

[84] Gestat de Garambé, 2000. Gestion des conséquences agronomiques et environnementales de la culture de betteraves tolérantes à un herbicide non sélectif. XIème Colloque International sur la Biologie des Mauvaises Herbes. Dijon, Septembre 2000.

[85] Gunn J.S., 1982. Population dynamics of weed beet. In: Proceedings 1982 British Crop Protection Conference-Weeds, Brighton, UK, 61-66.

[86] Boudry P., Mörchen M., Saumitou-Laprade P., Vernet Ph., Van Dijk H., 1993. The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. Theoretical and Applied Genetics, 87: 471-478.

[87] Longden P.C., 1993: Weed beet resurgence in 1993: the bolters return. British Sugar Beet Review vol 61, no 3.

> *species; infertile wild relatives; seed survival in relevant pedoclimatic conditions; the reproduction biology of the species; or other factors?*

192. From the above, it is clear that these plants which can establish survive and reproduce without any advantage would become invasive if an advantage was given to them.

193. The European Communities therefore agrees with Dr. Squire that:

> applying the herbicide would favour the HT plants, lead to greater seed set on HT plants and their increase in the seed bank.

194. The European Communities equally agrees with Dr. Snow when she states that:

> In the absence of exposure to the herbicide in question, herbicide-tolerant plants will not have any selective advantage over their non-transgenic counterparts. But when the herbicide is used repeatedly, it will select very quickly for plants that are resistant to the herbicide. The scientific literature in weed science is full of examples of rapid evolution and spread of herbicide resistant weeds.

195. It is important to note that spread of transgenes can occur not only via pollen, but by seeds and vegetative parts, and human activity most importantly. A model based approach[88] was used in a study dealing with the co-existence of GM and non-GM rapeseed crops in European Agriculture.[89] The aim of the Genesys-model[90] is to evaluate the effect of cropping system and rapeseed varieties on gene escape from rapeseed crops to rapeseed volunteers in neighbour plots and in subsequent crops.

196. Results of this study pointed out to the importance of border and set-aside management on the harvest purity,[91] as well as field spatial distribution (see below). Indeed, when borders and set-aside

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[88] The input variables of the model were (1) the regional field pattern comprising waysides, field edges and fields; (2) the crop succession of each field; (3) the agricultural practices used to manage each crop (stubble breaking, tillage, sowing date and density, herbicides, harvest conditions) and (4) rapeseed variety characteristics (genotype, self-pollination rates, differences between GM and non-GM varieties in pollen emission and yield). The main output variables are, for each year, adult rapeseed plants, newly produced seeds and seed bank. For each of these variables, the density of individuals and their genotypic proportions are given. The model describes the annual life-cycle of volunteer or cropped rapeseed plants, which is simulated for each plot and year. It comprises seed banks, seedlings, adult plants, flowers, pollen dispersal, newly produced seeds and seed dispersal. These stages depend on crop type and management. Pollen and seed exchanges between plots depend on plot areas, forms and distances as well as on flowering dates. The model is presently being evaluated. The first results show that it correctly ranks cropping systems according to their rapeseed volunteer infestation. However, pollen and seed dispersal is frequently underestimated and it was kept in mind when analysing the results.

[89] Bock, A.-K., Lheureux K,. Libeau-Dulos M. Nilsagard H., Rodriguez-Cerezo E., 2002. "Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture", Technical Report Series of the Joint Research Center of the European Commission, EUR 20394 EN., 133p.

[90] Colbach N., Clermont-Dauphin C., Meynard J.M., 2001a. GeneSys: A model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers. I. Temporal evolution of a population of rapeseed volunteers in a field. Agric. Ecosyst. Environ., 83, 235-253. Colbach N., Clermont-Dauphin C., Meynard J.M., 2001b. GeneSys-Rape, a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers. II. Genetic exchanges among volunteer and cropped populations in a small region. Agric. Ecosyst. Environ., 83, 255-270.

[91] Angevin F., Colbach N., Meynard, J. M., 2003. Coexistence of Rapeseed Varieties in Time and Space: Using GeneSys Model to Adapt Cropping Systems. 11th International Rapeseed Congress, Copenhagen, 6-10 July 2003, pp 732-734. Colbach N., Angevin F., Meynard J. M., Messéan A., 2004 Using the GeneSys

with feral populations (whether glyphosate tolerant or not) were treated by spraying glyphosate instead of mowing or cutting, rates of contamination in rapeseed harvests would be almost 15 times higher than the basic situation (current practices) giving evidence of the potential influence of feral populations on development of HT plants in non GM fields.

197. There is potential for the establishment and spread of GM HT crops, particularly for glyphosate (Roundup) or sulphonyl urea (eg Imidazolinone IMI) tolerant crops as these herbicides are widely used in agricultural systems for controlling weeds outside of the crop growing season, before crops have emerged and when they are used as crop dessicants. (See the reply of Dr. Snow on this). Also glyphosate is widely used on railway lines, road verges, parks, nurseries and gardens so that there is a potential for greater survival of feral HT plants and related HT wild species. However this is not so much of a problem with glufosinate (Liberty) as it is currently not widely used in agriculture except as a crop dessicant.

198. Resistance to the herbicides glufosinate and glyphosate is also an issue that has been raised in the procedures in dispute. Glyphosate is already one of the most widely used herbicides in the world and so how significantly the additional usage on HT crops would speed up the evolution of tolerant weeds is difficult to predict. Some resistance has already been found and some have argue that increased usage on HT crops will accelerate the evolution of resistance. This would initially be an agricultural problem but could be become an environmental issue if it results in an increased usage of herbicides with much worse enviromental profiles. (See also the reply of Dr. Snow). Farmers may have a range of alternative herbicides they can use to control HT volunteers or ferals, provided they know they are likely to be present.

199. It is noteworthy to consider the reply of Dr. Andow in his paragraph 06.15, as it touches upon an issue which has been discussed at length in the procedures in dispute.

200. His response does indeed point to an ambiguity in so far as HT crop plants may only partly be assessed for environmental impact, because the impact arises also through the application of the herbicide, which is often assessed separately. However, both components are to a large extent an in-separable package in the GM HT technology, and, as systemic issues may arise from the combination of both, some have argued that both should be assessed as a package. By separating them, some view that a component of the environmental impact may be excluded, and this may be at the heart of the confusion and, sometimes, unspecificity, of some requests for further risk assessment information. These requests were expressed by some that believed that assessing the environmental impact of HT crops without the effects of the corresponding herbicide may essentially be an incomplete, if not a moot exercise that might miss important aspects of the risk assessment.

201. It is also important to consider that one of the main agronomic advantages of HT systems is that you can use HT systems to control volunteers of the same species and closely related weeds in HT crops (e.g. weed beet in beet and wild turnip and radish in oilseed rape). If these species become HT themselves through gene flow then they revert to being problematic weeds in HT crops with a high potential to spread in the presence of the herbicide and to act as a reservoir of transgenes for other crops.

202. In beet seed crops there is currently an issue of crops being pollinated by wild or weedy beet. This results in annual beet seed that produces no harvestable root but which can grow as a weed in crops competing with them and shedding seeds to create further weed beet problems (see above). If

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model quantifying the effect of cropping systems on gene escape from GM rape varieties to evaluate and design cropping systems. OCL, 11: 11-20.

GM HT seed crops are pollinated by wild/weed beet then the annual beet contaminants would be HT and thus not controllable using the specific herbicide. This would then nullify a major benefit of HT beet is that the herbicide can be used to control weed beet.[92]

Infertile wild relatives

203. Infertile wild relatives are often difficult to control with existing herbicides which is why the HT systems may be attractive. However it will be important to manage the use of the herbicide so that resistant populations do not develop due to over use and selection pressure.

Seed survival in relevant pedoclimatic conditions

204. Seed survival is already high in many species (e.g. Brassica spp) and so provides a reservoir seed bank population to carry over contaminant GM into subsequent crops. Seed survival also depends on the regions in Europe where crops are grown. For example maize survives very poorly in regions with colder winters. But in warm and some maritime regions maize seeds and volunteer seedlings will persist and survive during winter and so could occur in subsequent crops or their field margins (see above).

The reproduction biology of the species

205. Outcrossing species (e.g. oilseed rape) are likely to disseminate genes more widely through cross pollination. Crops and weeds producing large quantities of small robust seeds (e.g. brassicae) also have better dispersal characteristics. (See comments above on cross fertility)[93]. However, the reproduction biology of crops may not always be well enough known. For instance, pollen dispersal of some major crop species is now known to be at a relatively constant density above a field over several hundreds of meters. This part of the atmosphere moves a lot and this results in very long distance dispersal of pollen. It took scientists a long time to realize this, because most of the pollen fall near the plant which produced them. But those pollen grain that do not can travel very far from their source. It was easy to find the pollen grains which did not migrate far, but it has been far more difficult to show the existence of the pollen at much further distances. This is done now.

Or other factors

206. Multiple physical and biological factors influence gene flow – (see reviews).[94] These may be biological factors: seed dormancy, seed longevity, seed vectors, pollen size, pollen viability, pollen vectors etc…, as well as physical factors: scale of GM crop, size of recipient crops, distance between donor and recipient crops, wind speed and direction, insect activity, air temperature, humidity, soil moisture and temperature , …

207. One expert addressed these issues in detail, and her line of argument is stringent and supported by science. But as of now, only the nearest steps of out-crossing to the next and most

[92] EASTHAM, K & SWEET, J B (2001) Genetically Modified Organisms: the significance of gene flow through pollen transfer. European Environment Agency, Environmental Issue Report 28, 75 pp.

[93] SWEET, J.B. (2003) Pollen dispersal and cross pollination. Proceedings of the First European Conference on the Co-existence of Genetically Modified crops with Conventional and Organic crops, Denmark. Danish Institute for Agricultural Sciences, 21-32.

[94] EASTHAM, K & SWEET, J B (2001) Genetically Modified Organisms: the significance of gene flow through pollen transfer. European Environment Agency, Environmental Issue Report 28, 75 pp. SWEET, J.B. (2003) Pollen dispersal and cross pollination. Proceedings of the First European Conference on the Co-existence of Genetically Modified crops with Conventional and Organic crops, Denmark. Danish Institute for Agricultural Sciences, 21-32.

compatible relatives (for example, *Brassica rapa* for oilseed rape) have been considered and researched in the context of GM plants. However, multiple transgene out-crossing events via several relatives of crop plants have not been studied in any great detail to our knowledge and none of the experts addressed this gap of knowledge.

208. All of the relevant scientific issues were theoretically known and discussed before and up to 1998[95], and such problems were all anticipated at that time by many experts but it had not happened yet at that time, since HT plants had only been recently released. However, similar processes were known from other cases before and that is why experts were anticipating them. However, the first peer-reviewed publication did not appear until early 2000s but it does in retrospect confirm those who did warn of these developments and re-enforce those who called for precaution. Today, Canadian farmers do have to carefully select the herbicides they can use and with no resistance management plan in place, this situation will worsen from year to year. For Europe and oilseed rape, the gene flow issue and its possible consequences for ecosystems (spread of transgenes, reservoir function, multiple pathways of spread) and agricultural practice (resistance issues, stacking, weediness, coexistence) are still being fully and rigorously thought through in all the consequences. The situation in Europe for oilseed rape is sufficiently complex, so that there remains uncertainties which are likely not to allow experts to get a full grasp of all the consequences in the limited time available.

(c) Third sub-question

> *(c) Is this potential different for biotech crops tolerant to two wide-spectrum herbicides? Please explain ?*

209. The European Communities notes that Dr. Squire implicitly calls for a case-by-case analysis:

> this will be highly specific to the agronomy of a farm or region

210. This question indeed depends mainly on the herbicides and the type of crop, e.g. in the case of oilseed rape (high potential to disperse and survive) with tolerance to herbicides widely used in cultivation, e.g. Imidazolinone (related to sulphonyl urea) and glyphosate (Roundup): then these have greater potential to become weedy and survive treatments in a range of farming situations. Herbicides from different groups to these would be needed to control them. However if the tolerance is to glufosinate and atrazine, which are only used in particular cropping circumstances, then weediness potential is generally less but could be a problem in certain farming systems dependant on these herbicides.

211. Only one of the experts, Dr. Snow, has pointed out the problem of stacking of different HT resistance genes in invasive crops, making it an increasingly uncontrollable weed within shortest period of time. At this time, triple resistant oilseed rape plants are confirmed in Canada.[96] These plants become increasingly difficult to control, and most certainly farmers have again to resort to 'herbicides which may have a negative environmental impact.

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[95] Rissler J & Mellon M (1996): The ecological risks of engineered crops. Cambridge, MA: MIT Press.

[96] Friesen LF, Nelson AG & Van Acker RC (2003): Evidence of contamination of pedigreed canola (Brassica napus) seedlots in Western Canada with genetically engineered herbicide resistance traits: - AGRONOMY JOURNAL 95(5): 1342-1347. Beckie HJ, Seguin-Swartz G, Nair H, Warwick SI, Johnson E (2004): Multiple herbicide-resistant canola can be controlled by alternative herbicides. - WEED SCIENCE 52(1): 152-157.

212. Generally speaking, the same type of potential impacts should be addressed; however, the magnitude of those impacts may sometimes differ significantly, as pointed out by Dr. Andow in his paragraphs 7.08 to 7.12 in his reply to the next question.

(d) Fourth sub-question

> *(d) If significant risks of establishment and spread have been identified, what risk management options exist to mitigate any resulting risks and what is their efficacy?*

213. The European Communities notes Dr. Squire's comment on risk management differences between GM crops and non-GM crops, depending upon the agricultural system, that:

> … need to be more stringently applied if cropping was to ensure the proportion of GM in non-GM remained below a threshold

214. The European Communities notes also Dr. Snow's comment:

> it is expected that transgenes that confer herbicide tolerance will not be associated with negative effects on the weedy plants that inherit them. This means that the transgenes could persist indefinitely in weed populations, even in the absence of exposure to herbicides.

215. Risk management options are obviously critical in this area, and the European Communities concur with the approach of the experts, and agrees that the listed measures could help reducing the risk of maintaining and spreading the HT genes, while noting however that they would have several shortcomings ecologically and agronomically. Knowing agricultural practice, compliance would be low and hardly to enforce. Not least of all, it would put quite a burden on the farmers, and potentially also increase the exposure of farmers to less innocuous chemicals, with all the related human health consequences, and it would not eliminate the risk after all.

216. The risk at stake relates mainly to farming and economics. Management may also include seed purity, segregation (temporal and spatial), labelling, separation of crops and products, careful recording of farm operations and management, crop hygiene, crop rotational management, careful planning of weed and volunteer control, monitoring of presence of HT volunteers and weeds etc.[97]

217. Properly adopted measures can be effective at minimising the building up of HT volunteers and weeds on farms. However they are liable to require changes to farming systems and have economic consequences. The measures for maintaining low levels of HT oilseed rape on farms will be the most difficult to implement and will require very stringent measures to maintain volunteers at low levels and restrict gene flow. Temporal separation of non-GM rape from GM rape will be several years (8-10) and spatial separation and sanitary measures will be needed.[98]

218. The European Communities will elaborate below on risk management options:

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[97] TOLSTRUP, K., ANDERSEN, S.V., BOELT, B., BUUS,M., GYLLING,M., HOLM,P.B., KJELLSON,G., PEDERSEN,S., OSTERGARD,H. & MIKKELSEN, S.A. (2003). Report of the Danish Working Group on the Co-existence of Genetically Modified Crops with conventional and organic crops. Danish Institute of Agricultural Sciences Report Plant Production No. 94 November 2003 275pp. Boelt B (2003) Proceedings of the First European Conference on the Co-existence of GM crops with conventional and organic crops, Denmark, November 2003. Danish Institute for Agricultural Sciences, 228 pp.

[98] SWEET, J. B., SIMPSON E. LAW., J, LUTMAN, P.J., BERRY, K., PAYNE, R., CHAMPION, G., MAY, M. WALKER, K., WIGHTMAN, P., & LAINSBURY, M. (2004). Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT Project). HGCA Project Report No 353, 265 pp. (www.hgca.com)

Case of sugar beets [99]

219. Weed beets are already a real problem in sugar beet fields as they belong to the same species as the cultivated form and are not sensitive to herbicides used in sugar beet crops. The technical institute in France estimated that one bolter in a sugar beet field leads to 18 bolters in the next sugar beet crop. In most European countries, situations have been observed in which sugar beet cultivation has been abandoned due to the yield losses inflicted by unmanaged weed beet infestations. Even in the first sugar beet crop of a field, specific practices are required to control bolters. Various aspects of the cropping system influence the evolution of weed beet populations:

– Bolter-specific practices, such as hand pulling in sugar beet crops, local herbicide application, cutting etc.;

– Weed management in the other crops of the rotation affect the weed beet seed bank between two successive sugar beet crops. For example, the seed bank decreases if groundskeepers and weed beet are eliminated by herbicides before crop production.

– The number of years between two sugar beet crops in the crop rotation also affects the weed beet population as weed beets produce their seeds mostly in sugar beet crops and the weed beet seed bank decreases in the other parts of the crop rotation.

– The choice of the appropriate sugar beet variety limits the appearance of weed beets, according to the number of hybrid seeds in the seed lot and the resistance of the plants to vernalisation.

Case of oilseed rape

220. From the available scientific evidence, it can be concluded that species like rapeseed has a potential for spreading and establishment as an HT crops. Risk management options would aim at achieving two main objectives: (i) the development or extension of practices aiming at reducing, in time and space, the persistence of undesirable plants (HT volunteers and hybrids with wild relatives) and (ii) the avoidance of selection pressure on these undesirable plants.

221. The establishment of GM HT oilseed rape can be reduced by:

– favouring the immediate emergence of seeds remaining on the soil after harvesting, in order to withdraw them from the seed bank: no tillage until the first rain then repeated minimum soil tillage avoiding seed dormancy;

– increasing the control of rapeseed volunteers within the subsequent crops in order to reduce the seed bank;

– not growing other crops resistant to the same herbicide within the rotation making it easier to control tolerant volunteers in the subsequent crops;

– organizing the spatial location of crops through adequate isolation distances and/or through regional specialization;

[99] Sester M., 2004: Modélisation de l'effet des systèmes de culture sur les flux de gènes entre culture transgénique et adventice apparentée. Cas de la betterave sucrière (Beta vulgaris L.). Mémoire de thèse de l'Université de Bourgogne. 153pp.

– and dedicating to tolerant crops the use of broad spectrum herbicides with their active matter used alone (glyphosate or glufosinate) and associating another active matter to these for their non-selective uses (pre-harvest applications, fallow land management).

222. As stated earlier, these measures as well as other measures such as feral plant control within edges, set-aside management or cropping systems changes, have been assessed, through simulations with the GENESYS®-rape modelling system.[100] Table 1 below illustrates the results for one specific situation. From this example, it turns out that three major factors must be taken into account when aiming at reducing the establishment of HT rapeseed plants: seed purity (effect of farm-saved seeds), management of fallow lands and road verges, and spatial distribution of rapeseed fields over landscape. Changing existing agricultural practices related to these factors will prove difficult to achieve as they would suppose a more integrated management of cultivated and non-cultivated areas (e.g. coordination between farmers and public road authorities) and could lead to additional costs or time requirements which are difficult to estimate as they will depend on each local farm structure and cropping systems.

[100] Angevin F., Colbach N., Meynard, J. M., 2003. Coexistence of Rapeseed Varieties in Time and Space: Using GeneSys Model to Adapt Cropping Systems. 11th International Rapeseed Congress, Copenhagen, 6-10 July 2003, pp 732-734.Bock, A.-K., Lheureux K,. Libeau-Dulos M. Nilsagard H., Rodriguez-Cerezo E., 2002. "Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture", Technical Report Series of the Joint Research Center of the European Commission, EUR 20394 EN., 133p.

Table 1. Effect of changes in cropping system on the level of impurities in hybrid seed production
Farm 1: 0% GM on farm - 50% GM outside
Typical rotation: rape/wheat/wheat/fallow/wheat/barley

GENESYS®-rape simulation	Relative impact on GM HT impurities
Basic system (Table 1)	1
Harvest loss in rape crops: 10% vs 5%	1.15
Farm seeds vs. certified seeds	4.025
Tillage before rape: plough vs. chisel	0.725
before other crops: plough vs. chisel	1.975
Rape sowing: early non-GM + late GM vs. simultaneous	3
late non-GM + early GM vs. simultaneous	0.275
Border cutting: mid-April vs. uncut	0.625
Border herbicides: glyphosate vs. none	14.9
Set-aside: spring sown vs. unsown	0.025
Rotation: spring barley added	0.425
Crop location: fields with past rape >200m vs.0 m	0.015
Farm field location: clustered vs. dispersed	0.025

223. According to some agronomists, it is always possible to control rapeseed volunteers and weed beets in cereal crops with "older" herbicides such as 2,4D (auxines), diquat, paraquat that are registered in European lists and some aminotriazol (not registered for this use yet). Profiles of these active ingredients are quite different from those of the new registered ones and it would be necessary to take into account differences in their environmental and human and animal health impact.

224. The European Communities note that Dr. Snow indicates:

> Management options become more challenging and more complicated when the pest population has genes for several types of herbicide resistance. In some cases, it may be necessary to revert to the use of herbicides that have greater toxicity and longer persistence in the environment (e.g., 2,4-D).

(e) Fifth sub-question

> *(e) What types of post-market monitoring and data collection activities could be envisaged on the basis of the monitoring and review principles described in ISPM 11?*

225. In ISPM 11, monitoring is primarily to identify novel or changed pest situations or to discover failures of pest management measures. Thus, according to ISPM11, post marketing monitoring could include monitoring populations of HT feral crop plants and weeds on farms to look for development of herbicide resistant populations. ISPM11 gives little emphasis on monitoring for environmental impacts, such as destruction of non-targets and beneficial organisms. However these are issues which have now become much more important in Integrated Crop Management systems as widely advocated throughout agriculture and one could argue that ISPM 11 is lagging behind in this respect (see in particular the recent FAO expert consultation on monitoring the environmental effects of GM crops referred above in the European Communities' comments).

226. The European Communities notes Dr. Squire's comments in that respect, on the difficulty of developing and implementing appropriate monitoring strategies, and the lack of details of ISPM 11:

> ISPM 11 does not give great detail on monitoring. The type of monitoring in this instance would differ depending on purpose: for example, monitoring the effect of a HT cropping on biodiversity or ecosystem functioning would require a different set of measurements from monitoring the presence or abundance of a HT trait. What is clear from existing data and work in progress is that monitoring of this type is far from simple and needs much time and effort, especially if the aim to measure low frequencies (e.g. 1%, 0.1%) or small effects (e.g. 1.5-fold effects on populations or ecological processes). Recently introduced populations are highly aggregated or clumped and this increases the number of samples and area over which samples have to be taken. At present, there are no reliable and accepted monitoring schemes for the presence or impact of biotech-derived plants: research is in progress to develop such schemes [ ], but it is not even certain that it would be feasible or practicable to monitor low frequency occurrences or ecological effects routinely in the field.

227. The European Communities would equally note Dr. Snow's comments in that respect:

> In cases where the persistence and proliferation of herbicide-resistant weeds could pose problems, post-market monitoring could be used to develop an early warning system for alleviating the problem. Weed scientists are already acutely aware of emerging problems with herbicide-resistant weeds that develop in response to heavy herbicide use. Many farmers are also familiar with these problems. Stewardship programs can be developed for risk management, for example by encouraging farmers to report cases of herbicide failure to the proper authorities. Studies of reported cases could be carried out to test for resistance due to transgenes, and to determine whether the stewardship programs are working as planned.
>
> However, by the time the spread of herbicide resistance is detected in free-living pest populations, such as weeds or volunteer plants, it may be too late to prevent these plants from proliferating further. By this time, continuous efforts may be needed to keep their populations as low as possible. After a transgene for glyphosate has spread widely in oilseed rape, as occurred in Canada, there is no easy way to eliminate the problem.

228. What is obviously needed most urgently, is a coherent resistance management strategy for weeds and their proper monitoring, similar to the efforts that went into developing strategies to mitigate insect resistance against Bt toxins. The European Communities agree with the experts, this task is far from being simple and cheap and quick. However with Bt plants, we have not yet seen significant resistance developing in insects; as with HT crops and glyphosate, we do see resistance developing in weeds. Once glufosinate resistant plants are grown on a similar scale as glyphosate-resistant crops, we can reasonably expect to observe developments also with them which might be of the same type.

229. As pointed out by Dr. Snow, the issue is to detect as early as possible the spread of herbicide tolerance in weeds and volunteers/feral populations. If one knows it exists, mitigation measures can be applied in order to minimize the appearance of such populations (see Snow and Squire above). That would certainly be the case for GM HT growers (although it has been observed that farmers not always comply with good agricultural practices recommended by companies or technical institutes).

230. However, due to pollen dispersal, such populations can also be present in non-GM farmer fields. Several studies have been carried out in various countries to assess the long distance pollen flow.[101] Over one kilometer distance, outcrossing rates are generally below 0.1 % but not equal to zero. As the number of seed losses at harvest is high (1,000 up to 6,000 seeds/m²), the presence of GM seeds in non-GM fields is not a rare event (around 1 GM seed per m² at long distance, thus 10,000 seeds/ha). In the absence of use of the specific herbicide, this will however represent a very small proportion of the volunteers (less than the outcrossing rate as seedbank already contains seeds).

231. Any application of the specific herbicide would led to inconvenience but not necessarily to a selection pressure:

– application of herbicide on volunteers (GM HT/non-GM HT) will leave GM HT volunteers alive but not always lead to seed production (e.g. pre-sowing spray of glyphosate before winter wheat sowing);

– the most sensitive compartments are fallow lands (if no use of mowing) and road verges and other non cultivated areas that could act as a reservoir.

232. This would mean that all farmers (both GM and non-GM) should comply with mitigation measures if we intend to mitigate the spread of herbicide resistance. This is technically feasible but not easy to implement under the current legal framework. This would also lead to extra costs for non-GM farmers.

233. The functioning of these field volunteer plant populations is now relatively well-known. A high volume of seeds (1000 to 6000 seeds per m^2 i.e. approximately 100 times the sowing dose) remains in the soil of the crop plot after harvesting. Once buried in the soil, these seeds remain viable, possibly for several years (between 5 and 10 years on average) and can re-grow in the rotation crops. Volunteer plants then represent a potential new source of pollen and seed emission. The number of volunteer plants per unit of surface area depends particularly on the moisture content of the soil and farming practices during the period between crops. The size and fertility of these volunteer plants decreases with the vigour of the farmed crops with which they are in competition.[102] Their frequency in subsequent oilseed rape crops appears to decline with the length of the rotation.

Question 7

On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that repeated use of a given biotech herbicide tolerant crop has adverse effects on flora and fauna, including soil micro- and macro-fauna? If so, how does this compare with any similar risks of adverse effects from the repeated use of a non-biotech herbicide tolerant crop (i.e, one developed through mutagenesis)?

[101] e.g. Rieger et al., 2002. SQUIRE, G.S., BROOKS, D.R., BOHAN, D.A., CHAMPION, G.T. et al. (2003) On the rationale and interpretation of the Farm Scale Evaluation of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B, 358, 1779-1800.

[102] Fargue, A.; Meynard, J. M.; Colbach, N.; Vallee, P.; Grandeau, G.; Renard, M., 2004. Contamination of rapeseed harvest by volunteers of other varieties: a study of intergenotypic competition. European Journal of Agronomy 21 (2): 193-207.

General comments

234. The European Communities notes that the Panel's question does not touch upon the use of different crops in rotation with the same herbicide tolerance, such as glyphosate or glufosinate, that are only used with GM HT plants, and its potential adverse effects.

235. The European Communities notes that two of the Panel's experts have addressed this issue (Drs. Andow and Squire). Dr. Squire's reply indicates that effects may be possible, and calls for a case-by-case assessment, but that further research is needed and ongoing, and that effects on biodiversity also relates to the effects of intensive agriculture. Dr. Andow agrees that there may be an effect in certain cases, and refers to the Farm Scale Evaluation study, but identifies as well that there are gaps in knowledge, as no study has been performed on soil micro-oganisms. Dr. Andow also indicates that GM HT crops are more likely to spread than conventional crops.

236. Dr. Squire has indicated in his reply, with respect to the Farm Scale Evaluation:

> The direction of effect differed depending on the severity of the conventional management. In maize, where conventional management uses persistent, highly toxic herbicides, GMHT increased the flora and fauna; in spring oilseed rape and beet, where current practice was less effective against arable plants, it had the opposite effect, reducing the flora and fauna. The crucial point about the FSE – and one that has been missed or misrepresented by many international commentators – is that the effects on the arable flora or weeds (though small by international standards) were important in the context of the UK's arable scene in the early 21st century.

237. The European Communities agrees that such effects have to be looked at for each GM crop, in each receiving environment.

Detailed comments

238. The repeated use of a given GM HT crop may have indirect effects through changes of farming practices. All agricultural practices constitute a significant and complex ecological disturbance, even if limited to a single and simple action. Indeed, whatever the nature and objective of the action, a large number of ecological processes are affected, and numerous discontinuities may occur within the agro-ecosystem, in both time and space.

239. Any change in technical practice (e.g. the introduction of GM crops, new conventional varieties, new chemical inputs, changes in the cropping system or in soil tillage) may therefore lead to changes in ecosystems, of various degrees of significance, through ecological processes and interactions with other agricultural practices. Such changes may have widespread environmental or socio-economical consequences. These changes affect not only the cultivated areas, but also the ecosystem as a whole and the natural environment at large. Furthermore, as successive operations within the field are interdependent, any change in one operation may necessitate modifications of various extents to the other operations or to the cropping system. The introduction of a new variety with a high level of resistance to a major disease may lead to decreases in chemical applications, or may even render chemical application entirely unnecessary. It may also make shorter rotations feasible. When assessing ecological impact, it is not sufficient to focus only on the direct effect of innovation; it is also necessary to take into account indirect and systemic effects.

240. As to HT crops, the risk of spread and establishment in agro-ecosystems is linked with changes in the practices associated with herbicide-tolerant transgenic crops and the use of the related herbicides. HT crops not only lead to a change of herbicide (with consequences on flora and fauna as

demonstrated in the FSE UK study) but also make easier other practices, such as minimum soil tillage or no tillage at all or lead to changes in cropping systems as observed in Argentina (substitution of grasses by soybean). Such indirect effects have environmental implications that could bear more impacts (positive and/or negative) than the direct effect of HT crops.

241. As this is likely to occur equally in European farming systems, an ex-ante evaluation of such indirect effects is a pre-requisite to set up appropriate risk mitigation measures as well as post-marketing monitoring programmes.

242. The ecologically adverse effects of HT tolerant crops may also arise significantly through the repeated large-scale use of the corresponding broad-spectrum herbicide, in particular those that can only be currently used on a large scale with GM HT crops. Other adverse effects could arise through unintended and unexpected changes in the GM plants metabolism through the process of genetic engineering, due to the expression of the transgene. That such effects can occur are known and have been documented in the peer-reviewed scientific literature.[103] Such effects can arise through the insertion of the transgene and depend on the position of the transgene in the plant genome, the neighbouring gene sequences, ectopic effects, gene silencing etc.

243. This is recognized by Dr. Andow, who states that:

> Most of the GM HT crops tolerate glufosinate and glyphosate, while most of the non-GM HT crops tolerate imidazolinone and sulfonylurea herbicides. It is possible that there are important differences in these herbicides that result in different risks to flora and fauna. I am unaware of studies that address this possibility. In addition, the options for managing resistant weeds are likely to differ among the different herbicides.

244. However, the European Communities disagrees with the statement of Dr. Squire in the last paragraph of its response, that "a general knowledge of the macro- and micro-fauna in the soil suggests they will be much less sensitive to GM HT cropping than will the above-ground flora and fauna". The European Communities believes that the level of knowledge in the scientific community does not exist to date to support that thinking. In fact, it might be the very lack of data and general neglect of soil ecosystems that might create the notion that soil systems are less sensitive. In the cases, where soil scientists look into it, they often find the very opposite. Soil systems react quite sensitively to chemical inputs, but because agricultural soils are already highly disturbed and often quite poor in biodiversity, and only productive with significant external inputs of agrochemicals, changes are not easily detectable, certainly not for lay people.

245. This is where this particular conclusion of this expert leads to a normative judgement rather than scientific ones. He acknowledges the widespread use of other agrochemicals and that in comparison to these, 'the herbicides glyfosate and glufosinate ammonium being much less directly toxic to soil fauna than other agrochemicals and the GM HT plants themselves'. While, it might be true that the two named herbicides are less toxic than say Atrazine, it is a normative judgement to imply that this would justify the more intensive use of a less toxic one. This may sound like a choice between two adverse situations, for which it is not clear whether one is better than the other. However, no long-term data (> 3 years) and systematically recorded field experience is available to

[103] Saxena D & Stotzky G (2001): Bt corn has a higher lignin content than non-Bt corn. - AMERICAN JOURNAL OF BOTANY 88(9): 1704-1706. Birch ANE, Geoghegan IE, Griffiths DW & McNicol JW (2002): The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (Solanum tuberosum). - ANNALS OF APPLIED BIOLOGY 140(2): 143-149

date on development of soil microflora and –fauna under large-scale use of GM HT crops and repeated applications of glufosinate or glyphosate.

246. Some data however, do emerge from the use of glyphosate resistant soybeans in the US and some of these findings do rather point in the direction of a change in soil microbial activity towards favouring fungi over bacteria. For example Kremer et al. (2000)[104] found that in soils repeatedly treated with glyphosate and grown to glyphosate resistant soybeans, soybeans significantly fell victim to a Fusarium fungus causing 'damping off'. It would in fact be rather surprising if such intensive use of one chemical would NOT cause a change in the microbial communities. The experience from Canada and the US also clearly show that the use of the respective herbicides complementary to GM HT crops do increase significantly with the production of the respective HT crops[105].

247. Finally, as regards the statement of Dr. Andow in his paragraph 7.05 that

> It may be possible that there are significant sources of genetic diversity in oilseed rape and beets in Europe.

248. The European Communities would like to provide some scientific evidence to clarify this issue, at least for the latter species mentioned.[106] Although the origin of the different species is not entirely clear, *B. napus* must be derived from cross between *B. rapa* and *B. oleracea*. Since the latter was originally confined to the Mediterranean region, it is believed that *B. napus* must have originated southern Europe from where it was introduced to into Asia in the early 18th century[107]. B. rapa is believed to be the oldest species and to have had the widest distribution. It could be found at least 200 years ago over an area extending from the west of Europe to the east of China and Korea and from Norway to the north of the Sahara and India.[108] Burkhill[109] proposed that *B. rapa* originated somewhere in Europe as a biennial form and that the annual form evolved later. Central Asia, Afghanistan or India may have bee another centre of origin.[110]

Question 8

What are the different detection methods currently available for testing for the presence of material from genetically modified plants?

(a) Have commercially available detection methods changed since the mid- 1990's? Were methods available in the mid-90's event specific?

(b) Please outline the steps necessary to validate a detection method, including the determination of what types of reference materials needed and differences in validation steps for qualitative and quantitative detection methods.

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[104] Kremer RL, Donald PA & Keaster AJ (2000): Herbicide impact on Fusarium spp. and soybean cyst nematode in glyphosate-tolerant soybean. American Society of Agronomy publication [online] Available from: http://www.biotech-info.net/fungi_buildup_abstract.html [Accessed 19 January 2005]

[105] C. Benbrook, 2004.

[106] Kimber D. S., McGregor D. I., 1995. The species and their origin, cultivation and world production. In "Brassica oilseeds. Production and Utilization", Kimber D. S., McGregor D. I. Eds, CAB International Publishing, pp 1-7.

[107] Downey & Röbbelen, 1989.

[108] Hedge, 1976.

[109] Burkhill, 1930 cited in Prakash, 1980.

[110] Sinskaia, 1928; Vavilov, 1949.

(c) What are the differences in the intended uses of qualitative and quantitative detection methods? What are the differences between event specific and non-event specific detection methods? How does the availability of different types of detection methods relate to risk assessment and risk management processes?

General comments

249. The European Communities regrets that only one expert has provided a reply to that important question. The European Communities notes that Dr. Nutti has provided some general, mainly descriptive, elements of reply, although in answering some parts of the question, she has stated that she is "not an expert in this area", and has limited herself to provide "the information that she could find" (she provides a list of references and of the information she found), "but that she did not express her personal opinion".

250. The European Communities will comment in detail below on the information she has provided, which is descriptive and factual, but would like to point already now that it does not agree with all her statements, such as the following one, which obviously contains an internal contradiction, that:

> *The protein based methods, while not event-specific, can identify uniquely most commercial events.*

251. The European Communities will therefore endeavour to provide further scientific and technical evidence to the Panel, in order for it to understand the reply of the expert and the state of play on detection methods as fully and objectively as possible. The European Communities has prepared this information with the assistance of scientists, experts in the field of the development and validation of detection methods.

252. The European Communities would also like to refer back to its initial comment in the section on general and methodological issues, on the relationship between detection methods of GM products, and risk management.

Detailed comments

253. Detection methods can be classified on the basis of the target, e.g. seeds, protein or DNA molecules. They can also be classified on the basis of their specificity relative to the genetic material they detect, e.g. screening, trait/construct and event specific methods. Finally, they can be classified on the basis of their ability to quantify or not the GM material. A comprehensive overview of detection methodologies and their applicability range and limitations is given in Miraglia et al. (2004).

254. Detection methods can be classified on the basis of the kind of material they can detect: the GMP itself may be detected by application of a wide range of methods, while processing will reduce the range of methods suitable for detection of GMP derived material. The by far most commonly applied currently available detection methods target either the modified genetic information itself, i.e. a DNA sequence, or a product of the modified genetic information, i.e. the transcript (an RNA sequence) or more often a translation of the transcript (a protein or an amino acid sequence). It is also possible to apply e.g. herbicide sprays to test for herbicide tolerant traits, but this is not widely applied.

255. Both DNA and some proteins are persistent to certain types of processing, and therefore both DNA and proteins may be detectable in processed material. Protein methods target particular

structures or epitopes of the proteins by application of immunological techniques; Western blots, ELISA (Enzyme linked immunosorbent assays), or lateral flow-strips. DNA methods target particular sequence motifs, usually by application of PCR (polymerase chain reaction) amplification of the specific DNA sequence followed by detection and/or identification of the amplification product(s). If DNA of high quality is available and from a low or unprocessed pure plant material, it is also possible to detect GMP derived material by digestion of the DNA with restriction enzymes followed by Southern bloting and hybridisation with a labelled probe corresponding to a particular sequence of interest.

256. With PCR detection/identification and quantification can be done simultaneously in real-time. Methods to detect GMP derived material may be classified into categories depending on their specificity.

257. Screening methods can detect material that is likely to be derived from GMP, but that may also be derived from a non-GM naturally occurring source such as soil bacteria or the Cauliflower mosaic virus (CaMV). These methods typically target commonly used regulatory sequences (e.g. the CaMV 35S promoter [P -35S] or terminator [T-35S], or the *Agrobacterium tumefaciens* nopaline synthase terminator [T-nos]), marker genes (e.g. neophosphinotrin transferase [nptII] or kanamycin [cam]), or parts of commonly applied cloning vectors (e.g. pUC18/pUC19 derived sequences).

258. Gene or trait specific methods can detect material from GMP or naturally occurring non-GM organisms containing a gene encoding the protein of interest. These methods typically target genes inserted in multiple GMPs (e.g. CryIA(b), EPSPS, Pat or Bar genes). Notably, the inserted genes may have been subject to modifications, so that the specific DNA sequence may be synthetic, in which case the detection method may be more specific (see also next category). Particular proteins or epitopes may also fall into this category.

259. Construct specific methods can detect material that is derived only from synthetic genetic constructs, e.g. a virus derived promoter in combination with a gene from one type of bacterium and a terminator from another type of bacterium.

260. Event specific methods can detect material that is derived from a single transformation event, e.g. the unique sequence motif created when an inserted genetic construct is fused to a recipient chromosome in the plant that is subject to genetic modification.

261. Notably, a regulatory sequence, gene or a genetic construct, naturally occurring or synthetic, can be inserted into a GMP in one or several complete or partial/truncated copies in the modified haploid genome. These sequences may therefore be considered unreliable with respect to their fitness for quantification purposes. Furthermore, these types of sequences can be introduced into more than one recipient plant, i.e. they may be found in more than one transformation event now or in the future. These sequences may therefore also be considered unreliable with respect to their fitness for identification purposes. In contrast, event specific target sequences are unique to a single transformed plant (the elite event) and its descending lines, and it is always present in only a single copy in the modified haploid genome. These sequences may therefore be considered reliable with respect to their fitness for quantification and identification purposes.

First sub-question

(a) Have commercially available detection methods changed since the mid- 1990's? Were methods available in the mid-90's event specific?

262. The answer to the first Panel Sub-question is yes, available commercial and non commercial detection methods have significantly changed since the mid 1990. DNA based methods available before then were not event specific; quantitative DNA based methods mainly applied competitive PCR compared to extensive use of real-time PCR, since the turn of the millennium. Finally, lateral flow-strip tests for detection of transgenic proteins were not available.

263. In the mid 1990, there were practically no methods commercially available for detection of GM plants, although restriction analysis combined with Southern bloting and DNA hybridisation was an option for molecular characterisation and detection, provided that a DNA probe was available (e.g. screening targets or gene/trait specific targets could be used since these were easily available).

264. The PCR technique was well established, but no suitable primers or probes were publicly available for the purpose of detecting GMP derived material, apart from those typically developed for amplification of sequences inserted into commercial cloning vectors. The first PCR methods developed specifically for the purpose of detecting GMP derived material were published late in the 1990's. The first method for real-time quantification of GMP derived material was published in 1999 and the first event specific PCR methods were published in 2000 and 2001 (see e.g. Holst-Jensen et al., 2003). In general, availability of detection methods has been restricted by lack of available sequence data and reference material required for method development and validation. There has also been significant developments, since the end of the 1990's of DNA isolation methods from various plant and food samples, in order to overcome technical barriers due to the presence of inhibitors of the PCR reaction, or to overcome the poor quality of the isolated DNA.

Second sub-question

> *(b) Please outline the steps necessary to validate a detection method, including the determination of what types of reference materials needed and differences in validation steps for qualitative and quantitative detection methods.?*

265. The European Communities agree with the explanation of Dr Nutti as regards the importance given to appropriate certified reference materials. For further information, it refers to documents available on the website of the Community Reference Laboratory[111].

266. Method validation is a very complex process, but simply outlined it consists of the following steps:

1. Method development and optimisation, including the determination of the method performance in relation to the scope of the method.

2. Method transferability studies (pre-validation trial in small scale).

3. Collaborative trial validation according to international harmonised standard and protocol.

267. First, it is necessary to define the term "method", because the term is used inconsistently. Relative to validation in the context of detection of GMP derived material, a method usually consists of a DNA extraction step and two PCR steps, one targeting a reference sequence (usually a single copy housekeeping species specific sequence) and the other targeting a GMP derived sequence. Sometimes the instructions associated with only one of these steps is referred to as a method. In the

[111] http://crl-gmo.jrc.it

following, a method will refer to the combination of instructions for all three steps. In the case of protein based methods there are usually only two steps involved, i.e. the extraction of protein and the detection/identification of the GMP derived protein. Protein based methods are usually applied semi-quantitatively, i.e. a qualitative test is performed with a sensitivity corresponding to a desired threshold. Because the vast majority of validated methods for detection of GMP derived material target DNA sequences, the following description of method validation is generally referring to how DNA based methods are validated (see also Miraglia et al., 2004).

268. **Method development and optimisation**. Method validation is a process starting with the method development on the basis of a particular scope and a particular reference material. The scope includes definition of the specificity (detection or identification), the sensitivity (lower limit of detection/quantification) and the application (matrix limitations, qualitative and/or quantitative).

269. After method development and optimisation, the performance characteristics of the method are described and evaluated. If the performance characteristics are deemed fit for purpose then it is strongly recommended to perform a small scale pre-validation trial including at least one or two laboratories familiar with the technology applied but not with the particular method. The purpose of this is to assess the method transferability prior to a full scale collaborative trial, in order to identify gaps in the protocol, sources of possible errors and to avoid wasting resources on sub-optimal methods.

270. **Method transferability studies.** The objective of the second step is to assess whether it is likely that the method will meet the desired performance values in a full collaborative trial. This phase includes preparative steps like selection of participants, production of reference materials and test samples, reagent quality testing, as well as evaluation and reporting of the resulting data.

271. Planning and preparing for the validation trial also includes deciding what kind of test and reference material is going to be used, and production of the test and reference material of appropriate quality in sufficient quantity. Test materials are the samples to be treated as unknown in the validation study. Reference materials are the samples to be treated as known references or calibrants in the validation study. Quality here refers to inherent characteristics of the material such as homogeneity, presence of the analyte of interest (e.g. DNA or protein) in a condition that allows for its detection and eventually for its quantification, and correctly assigned quantity of GMP derived material.

272. Most validation studies have used seeds or kernels as the basis for production of test and/or reference material, mixed on a weight: weight basis, and ground and blended to homogeneity. However, the grinding and blending process may affect the analyte of interest, e.g. by considerable degradation. Furthermore, a weight: weight based reference material may have an assigned GMP concentration considerably different from that determined analytically (e.g. a DNA: DNA based concentration). It is therefore important to ensure correspondence between the reference material and the test material, depending on the scope of the method.

273. If the method shall determine the weight: weight based concentration, then both the reference material and the test material should be made on the basis of weight units. If the method shall determine the analyte: analyte based concentration, then both the reference material and the test material should be made on the basis of analyte units. To further complicate, it is important that test material and reference material are produced independently. A problem with weight: weight based quantiation is that the same quantity can be obtained with the use of both homozygous and heterozygous seeds/kernels. Thus, if homozygous material is used to produce the reference material, and heterozygous material is used to produce the test material, a 50% measurement error will be introduced if the plant concerned is diploid and a DNA based detection method is applied. This

potential error is also relevant in the context of application of the method since it is not possible for the analyst to assess for a sample received the homo- vs. heterozygous status of its GMP derived material.

274. Therefore the most recent validation studies have focused only on the PCR steps, and prepared reference materials and test materials from purified DNA, in which the assigned concentration of GMP derived material is determined on the basis of target sequence copies. However, this also means that the DNA extraction step is somewhat detached from the method. This approach is referred to as the modular approach, and is currently subject to considerable debate. It is based on the assumption that DNA extraction is linked with the matrix, e.g. a flour produced from ground seeds/kernels, and that if a particular instruction for DNA extraction from e.g. a soya bean flour has been subject to successful validation then it may be applicable to other soya bean flour from substantially equivalent soya beans.

275. Presently, there are consequently two favoured options: either to 1) produce matrix based reference and test materials with assigned concentrations based on weight concentrations (which means there is a considerable source of error in the application of the method), or 2) produce analyte based reference and test materials with assigned concentrations based on analyte ratios (which means that if there is an analytical bias introduced by analyte extraction it is ignored erroneously).

276. **Collaborative trial validation**. This includes preparative steps like selection of participants, production of reference materials and test samples, reagent quality testing, as well as evaluation and reporting of the resulting data. The detailed listing of parameters etc. in Dr. Nutti's reply is indeed correct, but one of the major problems with GMO detection methods is to reach consensus on the values that must be achieved for each of the parameters for the methods to be considered fit for purpose. While the European Network of GMO Laboratories reached an agreement which was first published on the website of the Community Reference Laboratory in the summer of 2003, other bodies like the European Committee for Normalisation (CEN), TC 275/WG 11 and the International Standardization Organization (ISO), TC 34/WG 7, are still discussing on method acceptance criteria. Indeed the ISO WG recently proposed to establish a separate work item on defining method acceptance criteria in relation to the achieved specific values of the parameters in collaborative trial validation studies (ISO, 2004).

277. Prior to launching a validation trial all reagents must be quality checked, to avoid that impurities in e.g. bathces of PCR primers or probes compromise the result. Furthermore, it is critical to identify qualified and willing participants. A full scale collaborative trail must according to the internationally harmonised protocol for method validation (Horwitz, 1995) include at least 8 succesfull participants, and therefore it is common to include between 12 and 15 laboratories in a collaborative trail (buffering for non-replying participating laboratories and outliers). Inexperienced laboratories sometimes confuse method validation with proficiency testing and volunteer to participate in validation studies. To ensure that the method and not the laboratories are trialed, selection of participant laboratories is in itself an important task. The typical labour effort contributed to a method validation study by a participating laboratory, including reporting of data etc. is 2-5 person workdays per method. This may affect availability of volunteer laboratories.

278. On the basis of the defined scope of the method, the validation study is set up with test materials and reference materials covering a range of quantities of analytes. It is most common to consider the concentration of GMP derived material, and to prepare the materials to cover values in the range of 0% to 5% GMP derived material. However, the limit of detection and quantitation in PCR analyses is usually linked with the number of amplifiable copies of the target sequence. Recent validation studies therefore also consider the number of target copies in the test and reference

material. In this case the values may e.g. cover the range between 40 and 5000 copies for GMP specific DNA sequences and between 1000 and 100000 copies for the reference DNA sequences in each single PCR. Thus, the validation study may result in expression of method performance both in relative values (concentration, expressed in percent), and in absolute values (number of target sequence copies).

279. The planning, preparation and conduct of a validation study also includes defining how e.g. PCR plates are to be set up to determine the so called plate effect, the number of replicates and independent runs (plates) to determine the so called run effects and assess the repeatability standard deviation RSD_r. Data processing also includes treatment of outliers.

280. If a validation study shall also include assessment of the method specificity, this will require setting up negative target control samples. This is usually considered only in the context of method development. Method robustness is another parameter that is usually not subject to testing in collaborative trial validation. This would require testing the effect of intended modifications, e.g. altered reagent concentrations or altered temperature profiles in PCRs. This too is normally considered during method validation only.

Third sub-question

> *(c) What are the differences in the intended uses of qualitative and quantitative detection methods? What are the differences between event specific and non-event specific detection methods? How does the availability of different types of detection methods relate to risk assessment and risk management processes?*

281. Qualitative methods are used to assess whether GM material is present or not, and to identify the GMO from which the GM the material is derived, including assessment of its status as authorised or not. A qualitative detection method is typically intended for quick determination of the status of a material, i.e. does it contain or not a particular item (above the lowest limit of detection of the item)? Such a method would typically be used to assess whether e.g. unauthorised items are present in the material, or to assess whether further use of quantitative detection methods are required (e.g. in relation to authorised material and particular thresholds).

282. A quantitative detection method is typically intended for determination of the actual quantity of an item, e.g. in relation to a particular contractual or legal threshold. The unit of measurement plays a key role in defining quantitative methods and the resulting values of applying the methods. For example will a batch consisting of 100 GM maize kernels contain 100% GM if kernels or weight units are used, while it will contain between 40% and 100% GM if haploid genome equivalents [DNA copy numbers] are used, depending on the homo- vs. heterozygous status of each individual kernel, and whether the maternal and/or paternal gametes were GM or not.

283. Event specific methods can be used to establish with absolute certainty that a material is derived from a particular transformation event, and to quantify very reliably the haploid genome (DNA) based quantity of the derived material. Other methods like trait or construct specific methods may sometimes be used to establish with acceptable certainty that a material is derived from a particular transformation event, and to quantify with acceptable reliability the haploid genome based quantity of the derived material.

284. While event specific methods target a DNA sequence motif created as a unique consequence of a particular transformation event, all other methods (screening, gene/trait and construct specific) target DNA sequence motifs that may be present in more than one transformation event existing or

potentially created in the future. This is due to the nature of the transformation process itself (see e.g. Holst-Jensen et al., 2003).

285. Furthermore, in a haploid GM genome, each event specific sequence motif is present in only one copy, while any other sequence involved in the genetic modification may be present in more than one copy, and the number of copies may vary from one GMO to another. Consequently, event specific methods are ideal for identification and quantification purposes.

286. Two objections may be raised: that event specific motifs will also appear in stacked events, and that DNA based quantification may yield results significantly different from e.g. weight or seed number based calculations. Consequently, if stacked events are considered unique and should be distinguished from their parental counterparts, then there is presently no truly useful quantification method. And if weight or seed number based calculations shall prevail, then it is necessary to either test on single unprocessed seeds, or alternatively for processed material to know in advance the actual degree of homo- and heterozygous material, and the actual frequency of GM male and female germ cells that gave rise to the original material.

287. However, the reliability of event specific methods both with respect to identification and quantification purposes is superior to any other alternative. In relation to risk assessment and risk management, highly specific and quantitative methods can be very useful tools if a material is to be identified e.g. to be removed successively, in case of occurrence of unforeseen risks. To be able to develop an event specific detection method it is necessary to have access to the DNA sequence of the junction region between the insertion locus and the inserted DNA. This will include detailed characterisation of the inserted DNA, not just the original construct that was intended to be inserted.

288. This is important since it is well known that significant rearrangements of DNA in or associated with genetic constructs take place during transformation. And, equally important, this also implies that the insertion locus itself will be characterised. This in effect will allow for a better evaluation of the potential effects of the genetic modification, such as whether there is a risk of unintended genetic effects, and whether the copy number and location of insert is likely to be stable. Such data may also provide additional and valuable information about the different levels of predictability associated with different transformation technologies. Such information may later be used to give recommendations concerning the effectiveness and precision of alternative transformation technologies

289. However, sometimes, the gene product may be of particular relevance. Development of detection methods is often closely linked with molecular characterisation, and consequently may provide important background information to risk assessors.

Question 9

In what ways does molecular characterization inform the risk assessment for any particular biotech product? Can a risk assessment be carried out in the absence of a comprehensive molecular characterization of each transformation event?

General comments

290. The European Communities notes that all experts agree upon the fact that a molecular characterisation is useful and necessary in a safety evaluation. As rightly pointed out by all experts, this has been established in a number of international consensus documents and notably in the Codex Guideline CAC/GL 45-2003.

291. Dr. Andow emphasises that "a risk assessment cannot be carried out in the absence of a comprehensive molecular characterization of each transformation event." He considers it necessary for the prediction and verification of transgenic expression as well as genetic stability. Dr. Healy concludes that currently the molecular characterisation is an important element of safety assessment, providing verification that the intended genetic modification has occurred and information about unintended effects." Dr. Nutti confirms the "fundamental importance" of molecular characterisation for the food safety assessment of a GM product. From his perspective as an environmental expert, on the other hand, Dr. Squire considers that it is "not always" necessary when considering spread or persistence of GMOs or their effect on the ecosystem. He concludes, nevertheless that it is "reasonable" for an assessing body to request full characterisation.

292. Generally opinions tend to differ, however, as to the level of molecular characterisation that is needed for a safety assessment. There is now some guidance in international consensus documents as to what is reasonable and necessary to include on molecular characterisation, but the issue has been controversial throughout and remains in part controversial. Among scientists, on the other hand, there is considerable agreement on the kind of information required to sustain formulated conclusions. This fact is reflected in the experts' replies.

Detailed comments

293. The experts' general conclusion that a molecular characterisation is necessary is in line with Codex guideline CAC/GL 45-2003 which states in its paragraph 30.

> In order to provide clear understanding of the impact on composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

294. Molecular data provide insight into whether the intended modification has been achieved and is stably inherited, and also whether unintended effects may have occurred, for example by rearrangements of the inserted DNA[112].

295. This is part of the internationally acknowledged comparative approach of safety assessment of GMOs (also known as "substantial equivalence"). In this approach, the differences between a GMO and a conventional counterpart with a history of safe use are established. Based upon these differences, it can be decided upon which safety tests are further needed[113]. Molecular characterization will aid to clarify which changes have taken place in the GMO at a molecular level as caused by the genetic modification. Molecular characterization is therefore an essential part of the safety assessment of a GMO for environmental release, food and feed applications.

296. Dr. Squire's statement may cast a doubt on this generally agreed fact as he considers that molecular characterisation mzy "not always" be need for the purpose of assessing spread and persistence. First of all it should be pointed out that this statement is made from the specific perspective of an ecological/environmental expert. Indeed it may even have been made from the specific perspective of what is needed for the purposes of research rather than for the purposes of a safety assessment. Second, even if that were to be the case the European Communities is not quite in agreement with Dr. Squire on this point. To monitor spread and persistence of a new GM event, detection methodologies based on phenotypic trait(s), protein(s) and/or DNA are necessary. A

[112] See, for example, Kuiper, H.A., Kleter, G.A. (2003) The scientific basis for risk assessment and regulation of genetically modified foods. Trends in Food Science and Technology 14: 277-293.

[113] See also paragraph 13, of the Codex guideline CAC/GL 45-2003.

complete molecular characterisation forms the basis for all further monitoring tests. Especially in environments where different GM events may grow and occur next to each other.

297. Equally Dr. Squire's states that:

> for some aspects of comparing GM and non-GM plants, detailed knowledge of the molecular construct is not necessary. For instance, if comparing the ecological effects of GMHT or Bt plants, the studies could be done equally well without detailed molecular knowledge of the construct.

298. This statement is misleading. A GM plant could not be compared to a non-GM plant, if no molecular data at all were available about the inserted genes. Before the long-term ecological impact of e.g. a Bt plant can be assessed, detailed data are required on the exact structural composition of this Bt-transgene construct, e.g. the type of promotor (constitutive or not?), type of terminator, the type, length and exact part(s) of the Bt-gene(s) inserted, the copy number of inserted transgene construct and the number of insertion loci, and the stability of the inserted transgene construct copies. All these characteristics will influence the expression of the Bt gene(s) and thus the effect on ecology. E.g. one Bt-plant can have other ecological effects than another Bt-plant, depending on which Bt-gene(s) is(are) inserted, the construction of the T-DNA insert, the locus of integration, number of integrations and number of transgene copies per integration, and other genetic factors determining the expression of the gene(s).

299. There are differing views as to the level of detail for molecular characterisation needed. There is some guidance at the level of international consensus documents. Thus, the Codex Guideline CAC/GL 45-2003 recommends that

> a comprehensive molecular and biochemical characterization of the genetic modification should be carried out and information on the DNA insertions into the plant genome should include: A) the characterization and description of the inserted genetic materials; B) the number of insertion sites; C) the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.[114]

300. Furthermore, the latest FAO/WHO expert consultation on GM animals which updated the entire field in this area concluded that

> An extensive molecular characterization of the inserted genetic material construct will generally be required, both before and after the insertional event. The molecular characterization should furthermore *comprise an analysis of the copy number and a sequence analysis of the flanking regions of the place of insertion in order to identify any unintended effects* [emphasis added][115]

[114] Codex Guideline CAC/GL 45-2003, para. 31.

[115] FAO/WHA, 2003 in Transgene Locus Structure and Expression of Bt Maize, Environ. Risk. Chap 04 13/5/04.

301. In the scientific literature, this need has been confirmed. Thus, Andow et al.**,** recommend that

> DNA sequencing is necessary to characterize the fine structure of the transgene locus and its flanking genomic region. With sequence information, it is possible to identify rearrangements that may produce unexpected protein products, to design probes necessary to establish the presence or absence of unexpected RNA or protein products, to evaluate the risk of combinations or other rearrangements, and to evaluate the possibility that plant gene expression is disturbed. *None of the other methods for characterizing transgene locus structure can enable assessment of all of these potential risk.* [emphasis added][116]

V. THE PANEL'S QUESTIONS ON ISSUE 1 ("DELAY") (NOS 10 TO 58)

Bayer oilseed rape (Falcon GS40/90)
C/DE/96/05 (EC chronology 62)

Question 10

Given the information before the Panel, including the notification by AgrEvo (EC-62/At.1-30) and the EC SCP's opinion (EC-62/At.74), was the information to assess the long-term effect of the newly expressed protein on the biogeochemical cycle and the food chain requested by the Italian CA (EC-62/At.95) necessary to ensure that conclusions of the safety assessment were valid?

General comments

302. Two Panel's experts have answered this question, Dr. Nutti and Dr. Andow. Although Dr. Nutti focused on safety assessment in the food chain and Dr. Andow analysed the environmental aspect, their assessment of the scientific evidence submitted by the applicant differs substantially. Dr. Nutti considers it "sound scientific evidence".[117] Dr. Andow points in several instances to the limited scientific value, or to the absence of, scientific studies.[118] The European Communities finds that Dr. Nutti's response, by not reviewing the evidence actually in front of the SCP and by not citing and discussing any relevant scientific evidence, presents a conclusion which is not only erroneous but also unmotivated. Dr. Andow, on the contrary, critically evaluates the available information and presents his own conclusions. Nonetheless, the European Communities does not fully share all of Dr. Andow's reasoning and conclusions.

Detailed comments

303. In its only paragraph on this question, **Dr. Nutti** agrees with the conclusion of the SCP that "the protein was safe for human consumption in the food chain and was based on sound scientific evidence, which was presented by the applicant".

304. The 'sound scientific evidence' to which Dr. Nutti refers consists only on checking protein homologies with existing allergens and a study of the isolated purified transgene product in a simulated gastric fluid.[119] Based on this, Bt proteins are degraded within minutes. However, today we

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[116] Andow, D.A., Somers, D.A. Amugune,N., Aragao, F., Ghosh,K., Gudu,S., Magiri.E., Moar W.J., Osir (2004). Environ. Risk., Chapt 4 in CAB International 2004. Environmental Risk Assessment of Genetically Modified Organisms: Vol. 1. A Case Study of Bt Maize in Kenya (eds A. Hilbeck and D. Andow).

[117] Answers by Dr. Nutti of 7 January 2005, p. 12.

[118] Responses to Questions Submitted by Dr. Andow of 6 January 2005, para. 10.03, 10.04, 10.09.

[119] See, SCP Opinion, (EC-62/Att.74).

know that when embedded within transgenic plant material Bt-proteins can pass even through the intestinal tract of cows.[120] The request made by the Italian authority was, therefore, well founded. In reality, some experts consider that the data requested by the Italians on food chain should become routine information provided in approval documentation.

305. In **paragraph 10.08** of his response, **Dr. Andow** concurs with the finding of the SCP at that time, that the provided information was 'not scientifically convincing that the environmental effects of Falcon GS 40/90 are in no way different from non-transgenic cultivars.' Plot sizes were apparently so small that no statistical analyses could be conducted by the notifier and, thus, could hardly detect differences anyway. The European Communities considers correct to conclude that the scientific value from such small studies is rather limited. Therefore, it is appropriate to request more data from the notifier which the notifier in turn did on relevant aspects of the risk assessment, 'dispersal of OSR pollen', the 'potential invasiveness' and 'a number of aspects related to fitness, weediness and out-crossing' – although the expert does note that neither the data nor the sources were provided which is crucial for an independent assessment.

306. In **paragraph 10.09**, Dr. Andow notes the contradiction of the SCP which then concluded that there is no evidence to indicate that Falcon GS 40/90 is likely to cause adverse effects on human health and the environment while admitting that only 'few studies have been conducted on the safety of modified OSR to other organisms' – and no studies on geochemical cycling or the food chain were done.

307. In **paragraph 10.10**, it is quite appropriate of the expert to point out at this point that 'lack of evidence does not imply that there is a lack of an effect.' Statements on 'no evidence to indicate … adverse effects …' clearly need to be substantiated more than just basing it on lack of studies. This is not satisfactory from a scientific committee. Such a conclusion is not logically supported by the provided information.

308. **Paragraphs 10.13** and **10.14** relate to the problem of unspecified requests by the CA and insufficient information delivered in turn by the notifier. The European Communities agrees that the request from Italian CA is rather general and thus difficult to address but Dr. Andow clearly states that food chain and biogeochemical had not been addressed by the notifier.[121] Therefore, the European Communities disagree with its conclusion that "unless the Italian CA can point to prior regulator precedents where these terms are clarified, the phrasing of the request is not scientifically justified".

309. With regard to the comment by Dr. Andow, contained in **paragraph 10.14**, on the length of time it took the Italian authorities to review the information received and to pose new questions, the European Communities refer the Panel to its ***General comments*** above.

Question 12

Given the information before the Panel, including the notification by AgrEvo and the conclusions of the EC SCP, was the information regarding the molecular characterization of this product requested

[120] Chowdhury EH, Shimada N, Murata H, Mikami O, Sultana P, Miyazaki S, Yoshioka M, Yamanaka N, Hirai N & Nakajima Y (2003): Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves, VETERINARY AND HUMAN TOXICOLOGY 45, pp. 71-75; Einspanier R, Lutz B, Rief S, Berezina O, Zverlov V, Schwarz W & Mayer J (2004): Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgenic maize, EUROPEAN FOOD RESEARCH AND TECHNOLOGY 218, pp. 269-273.

[121] See paragraph 10.09.

by the lead CA (EC-62/At.106) necessary to ensure that conclusions of the safety assessment were valid?

General comments

310. Two Panel's experts have answered this question, Dr. Healey and Dr. Snape, reaching opposite conclusions. The two experts seem to address the question in a different way. While Dr. Healy addresses this question more from a historical perspective, Dr. Snape takes into account the current state-of-the-art. In fact, the dossier was submitted in 1996 and at that moment, molecular characterisation was focused on the inserted DNA fragment. However, the European Communities cannot concur with Dr. Healy because already then, (1) a number of scientists evaluating the dossiers insisted for more information on the molecular characterisation and were convinced of the value of this information for safety assessment;[122] (2) the RoundupReady soybean case was known and could have been taken into account;[123] and (3) it had already been proven that, technically, it was possible to carry out the experiments needed to address the question of the leading CA in 2002.[124]

311. For what concerns safety assessment, the European Communities wants to stress that a safety evaluation of a new event cannot be called 'complete' as long as some aspects of molecular characterization, such as the screening for inserted fragments and the sequencing of the plant genome DNA flanking these insert(s), are missing. In this case, no definitive statement can be done on the absence of rearrangements and unintended effects, and thus neither on the safety of the product. The European Communities, therefore, notes that some experts, such as Dr. Snape, are of the opinion that "more information is still required", which obviously necessitates further clarification and information before the issue can be definitively resolved. Some specific comments on the responses are provided below.

Detailed comments

312. In the first paragraph of page 5, Dr. Healy states that:

> The molecular characterisation submitted included a sequence analysis of the *pat* gene isolated from the soil bacterium, full DNA sequence analysis of the DNA construct containing the *pat* gene and Southern blot and Polymerase Chain Reaction (PCR) analysis of the transgene. The latter demonstrated that the *pat* gene was stably inserted into two separate locations within the recipient strain and that vector sequences outside the border regions had not integrated.

This factual assessment is wrong. In reality, the information (EC-62 At.3, 13, 14) provided before 2 April 2002 (date of request for additional information, see EC-62/At.106) failed to identify the exact

[122] Windels P, Theuns I, Dendauw J, Depicker A, Van Bockstaele E, De Loose M (1999), Development of a line specific GMO detection method: a case study, Med. Fac. Landbouwk. Wet., 64/5b, pp.459-462. Windels P, Depicker A, Van Bockstaele E, De Loose M (2000), Characterisation of the 3'NOS junction of Roundup Ready soy, Med. Fac. Landbouww. Univ. Gent, 65/3b, pp.463-465;

[123] Windels P, Taverniers I, Depicker A, Van Bockstaele E, De Loose M (2001), Characterisation of the Roundup Ready soybean insert, Eur. Food Res. Technol., 213, pp.107-112.

[124] Depicker A, De Buck S, Van Montagu M, Theuns I, Huyghebaert A, Van Bockstaele E, De Loose M (1998), Detection and characterization of transgene inserts into plants, Med. Fac. Landbouww. Univ. Gent, 63/4b, pp.1479-1484; Theuns I, De Buck S, Depicker A, Van Gaver D, Huyghebaert A, Van Bockstaele E, De Loose M (1998), Anchored PCR method as alternative to detect and identify GMOs, Med. Fac. Landbouww. Univ. Gent, 63/4b, pp.1539-1542; Theuns I, Windels P, De Buck S, Depicker A, Van Bockstaele E, De Loose M (2002), Identification and characterization of T-DNA inserts by T-DNA fingerprinting, Euphytica, 123, pp.75-84.

number of copies of transgenic sequences into GS40/90. Initially, two intact copies of the *pat* gene construct were believed to be inserted. Later, as correctly noted by **Dr. Snape,**[125] it was determined, following the requested information, that the total copy number of the *pat* gene was shown to be three.[126] According to the European Communities, this underlies the need for complete molecular characterization from the beginning and justifies the CA request for more information on this topic.

313. In the last paragraph of page 6, Dr. Healy states that:

> *Impact of the Additional Information on the Conclusions of the Safety Assessment*
> The additional information requested by the Lead CA is unlikely to influence the conclusions of the safety assessment carried out by the SCP.

The European Communities submits that this would only be the case if no deletions, insertions or any other rearrangements in the flanking plant genome DNA had occurred. However, as no sequence information was submitted originally on the genomic DNA adjacent to the insert, but only on the inserted genes, at that moment, no evidence could be given of the absence or rearrangements. As long as no evidence is given on the absence of rearrangements, also outside the inserted construct, no conclusions on the safety of the event can be drawn.

314. In the following bullet-points on **pages 6 and 7**, **Dr. Healey** gives the rationale for her conclusions. In the **first bullet-point**, she affirms that, as "the transgene was inserted into the Falcon GS40/90 line via agrobacterium mediated transformation techniques, this technique is less likely to result in DNA rearrangements and other modifications of the inserted DNA". The European Communities disagrees. There is substantial evidence in literature that Agrobacterium-mediated transformation induces severe changes in the plant chromosomes and therefore may be the cause of many unintended effects. In fact, truncations, rearrangements, etc., cannot be avoided with Agrobacterium-mediated transformation.[127]

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[125] Report by Dr. Snape of 5 January 2005, p. 1, point (1).

[126] The information (EC-62 At.3, 13, 14) provided before 2 April 2002 (date of request for additional information, see EC-62/At.106) shows that oilseed rape event Falcon GS40/90 was transformed using plasmid pHoe6/Ac by means of Agrobacterium-mediated transfer. The T-DNA of the plasmid pHoe6/Ac carries a pat gene construct conferring resistance to the herbicide glufosinate ammonium. Southern Blot analysis indicated that two intact copies of the pat gene were inserted at two individual functional loci (At.14). To prove that no region outside the two border sequences of the inserts was present, Southern Blot analysis was conducted. However, the Southern Blot analysis undertaken seems to have been conducted on an oilseed rape line containing only one copy of the pat cassette (At. 113). The additional information (EC-62 At. 114) requested by the lead CA on 2 April 2002 gives more information on the transgene loci, denoted EE1 and EE2. It is said that (data proving this are lacking) one of the two functional loci contains two pat expression units in inverted tail-to-tail orientation, bringing the total copy number of the pat gene to three instead of two. The sequence of locus EE1 and its 5-prime and 3-prime flanking sequences, together with the DNA sequence of its pre-insertion site were determined. For locus EE2, no sequence data were given on the insert itself, but the 5-prime and 3-prime flanking sequences, together with the DNA sequence of its pre-insertion site were determined. Sequence analysis of the EE1 locus showed that this sequence contains one T-DNA copy and lacks the 65 sub-terminal bases at the RB and ends at the break point of the left border. A BLAST search on the 5-prime and 3-prime flanking sequences of both transgene loci revealed no meaningful similarities. Alignment of the 5-prime and 3-prime flanking sequences of the transgene loci with the DNA isolated from wild type oilseed rape revealed minor and major deletions and the presence of filler DNA. For EE1, a fragment of 1715 bp was deleted upon integration of the T-DNA. In addition, at the 3-prime junction of EE1, eleven nucleotides 'filler' DNA were inserted at the transgene locus. Sequence alignment with EE2 revealed a loss of 16 nucleotides at the target site.

[127] Windels P, De Buck S, Van Bockstaele E, De Loose M, Depicker A (2003), T-DNA integration in Arabidopsis chromosomes. Presence and origin of filler DNA sequences, Plant Physiology 133, pp.2021-2068.

315. In the second bullet-point, Dr. Healey asserts that

> The molecular characterisation originally submitted did include a PCR analysis, which indicated that only the DNA sequences associated with the *pat* gene had integrated into the recipient strain. In particular, the analysis verifies that that no sequences from the vector, others than intended, integrated into the recipient strain.

Now, PCR analysis of inserted genes only cannot provide any information on rearrangements, truncations, presence of vector backbone DNA, etc. which occurred outside of the amplified genes. This highlights the importance of complete molecular characterization, including sequencing of the complete T-DNA insert and adjacent plant genome sequences, and thus justifies the additional request of the lead CA. Also, as seen above, the information on molecular characterization submitted after additional request by the CA was *different* than the molecular data originally submitted. Therefore, as some aspects were not *known originally*, some aspects of the risk assessment were missing at the moment of the risk assessment.

316. As with regard to **Dr. Healy's conclusion** that:

> The additional molecular characterisation requested by the Lead CA is unlikely to affect the conclusions of the safety assessment and was not necessary to ensure that the conclusions of the safety assessment were valid.[128]

The European Communities would like to note that, if no sequence data of the T-DNA/plant junction regions are available, no evidence can be given of the absence of any rearrangement at the locus of integration in the plant, and thus neither of the absence of any unintended effect and thus ecological impact. As long as this evidence is lacking, the risk/safety assessment of the event cannot be considered as being complete.

Question 13

Given the information before the Panel, including the notification by AgrEvo and the conclusions of the EC SCP in relation to the potential persistence or invasiveness of Bayer oilseed rape (Falcon GS40/90), would this product qualify as a potential "pest" as the term is used in ISPM 11?

General comments

317. Four Panel's experts provided a response to this question: Dr. Andow, Dr. Nutti, Dr. Snow and Dr. Squire. Dr. Nutti states, without much of an explanation on why, that Bayer oilseed rape (Falcon GS40/90) is not dangerous and thus cannot qualify as a "pest". Both Dr. Snow and Dr. Squire, on the contrary, recognise that Bayer oilseed rape has the potential to be a "pest", but Dr. Snow adds that other methods of weed control could manage this problem. Dr. Andow considers that the information in front of the Panel, both as submitted by the notifier and as contained in the SCP opinion, are insufficient to determine the "pest" status of this product.

See also, for instance, Genome Scrambling - Myth or Reality?; Transformation-Induced Mutations in Transgenic Crop Plants by Allison Wilson and Jonathan Latham and Ricarda Steinbrecher available at <http://www.econexus.info/pdf/ENx-Genome-Scrambling-Summary.pdf> (last visited on 25 january 2005):

"This report identifies the insertion-site and genome-wide mutations created by plant transformation procedures as potentially major, but poorly understood, sources of hazard associated with the production and use of commercial transgenic cultivars".

[128] Responses by Dr. Healy of 6 January 2005, p. 7.

318. For its general view on the persistence of HT products and their potential as "pests", the European Communities refers the Panel to its comments under Question 6 above. With regard to the specifics of this question, the European Communities considers oilseed rape (OSR) a crop with weedy potential, especially in other broad leaved crops in arable rotations. Introduction of herbicide tolerance into OSR further reduces options for managing it and thus it could be argued that, under certain circumstances (e.g. existing use of the specific herbicide), its pest or weed status is increased by the introduction of HT traits and requires additional measures to manage it. Likewise some related species are also weeds and their weediness could be enhanced by the introduction of HT traits via gene flow. The European Communities thus concurs with Dr. Snow's acknowledgment of the fact that specific measures should be taken to control a "potential pest" and that "management options become more challenging and more complicated when the pest population has genes for several types of herbicide resistance".[129] Furthermore, as this "potential pest" could spread out of the GM farms, control should also be applicable by non-GM farmers in order to be efficient. However, some of these management options may be difficult to implement (especially in non-GM farms), have higher costs and/or less desirable environmental impacts.

Detailed comments

319. As said in the comments under Question 6 above, all crops are weeds to a greater or lesser extent when they grow in other crops. Oilseed rape has many characteristics of a weed: high seed set, high seed dormancy, variable germination, competitive under fertile growing conditions, etc. In addition GMHT rape has the ability to grow in subsequent non-GM rape crops even when grown after several years interval, resulting in potential impurity problems[130]. In addition if the HT gene transfers to existing related weeds, they in turn can become problematic weeds of the HT crop since the specific herbicide will not control them.[131]

320. Glufosinate is currently used as a herbicide and a dessicant in certain crops (e.g. potato). Introduction of glufosinate tolerant oilseed rape will mean that glufosinate cannot be used to control HT rape volunteers in these crops. Glufosinate is also widely used as a dessicant in conventional oilseed rape crops. This practice would have to change in order to manage conventional crops containing admixtures of glufosinate tolerant crops. In addition another dessicant would be required on glufosinate tolerant oilseed rape crops.

321. Glufosinate tolerance has been developed in maize and sugar beet. Glufosinate tolerant oilseed rape would be a problematic weed in these crops requiring additional weed control measures.[132]

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[129] See also Responses to Scientific Questions from the Panel of Dr. Snow of 5 January 2005, page 14, question 6d.

[130] SWEET, J. B., SIMPSON E. LAW., J, LUTMAN, P.J., BERRY, K., PAYNE, R., CHAMPION, G., MAY, M. WALKER, K., WIGHTMAN, P., & LAINSBURY, M. (2004). Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance in winter oilseed rape and sugar beet (BRIGHT Project). HGCA Project Report No 353, 265 pp. Available at <www.hgca.com>.

[131] Norris, Carol, Jeremy Sweet, John Parker & John Law (2004) Implications for hybridization and introgression between oilseed rape (Brassica napus) and wild turnip (B. rapa) from an agricultural perspective. In: Hans C.M. den Nijs, Detlef Bartsch & Jeremy Sweet (Editors 2004) Introgression from genetically modified plants into wild relatives and its consequences. Proceedings of an ESF AIGM conference, Amsterdam, 21-24 January, 2003, pp. 107-124.

[132] See Sweet et al (2004), cited above. MESSEAN, A. (2002). Monitoring case report: impact of transgenic plants within cropping systems. In LMO's and the Environment: proceedings of an international conference. November 27-30, 2001. Raleigh, USA. Edited by Craig Roseland. pp 207-215.

322. All of these above issues were not raised by the SCP in its Opinion (EC62/Att.74) who focused very much on whether the HT rape would be a problem in crops other than oilseed rape or other HT crops. However the SCP did comment that glufosinate tolerant rape should not be grown in proximity to rape with other HT traits, since combination of HT genes in a single rape plant might make it harder to manage. This is based on the Canadian experiences of gene stacking referred to in Dr. Snow's submission.

323. SCP also commented that: Glufosinate drift into field margins may enhance establishment of feral HT rape populations in these areas. This conclusion may have been based on the report by Sweet et al[133].

324. Although most of these above issues are manageable by changing agronomic practices and using different herbicide programmes, some of these changes (e.g. extending crop rotations between HT and non-HT crops) may not be economically justified, environmentally desirable or even difficult to implement (changes in non-GM farms).

325. Furthermore, the SCP qualified its response by saying that oilseed rape is a relatively new crop in Europe, information on weediness, gene flow and invasiveness (especially of related species) is limited and that caution and close monitoring is required.

Bayer hybrid oilseed rape (MS8/RF3)
C/BE/96/01 (EC chronology 63)

Question 15

Given the information before the Panel, including the conclusions of the EC SCP, was the information regarding the assessment of the long-term effect of the newly expressed protein on the biogeochemical cycle and the food chain requested by the Italian CA (EC-63/At.87) necessary to ensure that conclusions of the safety assessment were valid?

General comments

326. Two Panel's experts answered this question: Dr. Andow and Dr. Nutti. As under question 10, where the issues at stake were similar, their conclusions diverge substantially, on the basis of the same information before them. While the lack of evidence – no additional evidence was put forward in addition to the limited amount contained in the notifier's documentation – was interpreted as indication for safety by Dr. Nutti, Dr. Andow stated correctly that it is not sound science to interpret lack of evidence as evidence for lack of an effect and even less so as evidence for safety. While this may seem subtle, it is yet of great bearing for the safety discussion and often the reason for dissent.

Detailed comments

327. **Dr. Nutti** focuses on the bar-gene and PAT gene expression and transgene product. She concludes that since the bar-gene is controlled by plant promoter, it would not be functional in a bacteria. "Consequently, its expression in the unlikely event of transformation would not occur"[134]. While Dr. Nutti may be correct to state that in the unlikely event of horizontal gene transfer of a bar-gene, PAT product expression might not have negative effects as long as the substrate, the herbicide

[133] Sweet J.B., Shepperson R., Thomas J.E., Simpson E. (1997) The impact of releases of genetically modified herbicide tolerant oilseed rape in the UK. Proceedings of the Brighton Crop Protection Conference - Weeds. pp 291-302.

[134] Answers by Dr. Nutti of 7 January 2005, p. 14, first paragraph of Answer 15.

glufosinate, is missing. However, the argument that the bar gene would not be expressed because the plant promotor controlling this transgene in the plant would not work in bacteria is insufficient. The bacterial promoters can be expected to be able to do the job even better than the plant promoters. Expression of a transferred transgene of former bacterial origin cannot be excluded in bacteria.

328. Further, the expert speaks of "weight of evidence available elsewhere concerning the safety …"[135], however the evidence is missing and no references are provided. Arguments like these can often be heard in the discussions about safety of GM food and products. However, when researching for the 'weight of evidence' one finds more often than not that this evidence is actually meagre, unpublished, informal, contained in grey literature or notifier documentation. If such points become crucial for the case, the weight of evidence should actually be requested to be put forward in verifiable form.

Question 16

Given the information before the Panel, including the conclusions of the SCP, was the information regarding molecular characterization of this product requested by the lead CA (EC-63/At.107) necessary to ensure that conclusions of the safety assessment were valid?

General comments

329. Two Panel's experts have answered this question, Dr. Healey and Dr. Snape, reaching different conclusions. While for Dr. Healy the lead CA had enough information to conclude on the safety of Bayer hybrid oilseed rape (MS8/RF3) already in 2002, according to Dr. Snape "more information is still required".

330. The European Communities general comments on the experts' replies on question 16 are the same as those made on question 12, with the difference that here the missing molecular data – that were requested by the lead CA in 2002 – concern the comparison between the GM event and the isogenic line. Thus, complete characterization of GM events at the molecular level, including a comparison at DNA level between the insertion locus in the GM event and the corresponding locus in the isogenic line, gives detailed and highly valuable information on (1) the exact composition of transgene inserts, (2) the exact number of inserts and number of transgene copies per insert (including inverted repeats of a transgene copy at one site of integration), (3) the presence of vector backbone DNA sequences within the insert or outside the insert, (4) the occurrence of deletions, insertions or any other rearrangement within the transgene insert(s) and/or the adjacent plant genome DNA, (5) the stability of transgene inserts. Unexpected and unintended effects of transgenes cannot be excluded without detailed molecular data on all above mentioned topics. Some specific comments on the responses are provided below.

Detailed comments

331. With regard to molecular characterisation data, in the **first and second paragraph of page 9**, **Dr. Healy** concludes that no homology with known sequences was found using the 3' and the 5' flanking sequences. The fact that no sequence homologies with known sequences were found for the 3' flanking sequence of MS8 and the 5' flanking sequence of RF3, and the fact that the sequence data of T-DNA/plant junction regions have not been compared to the isogenic line, cannot exclude that unexpected and unwanted truncations or rearrangements occurred at these sites of integration. Providing this type of information gives the CA a more comfortable position to come to a positive evaluation of the safety. Absence of this information does not necessarily lead to a negative report.

[135] Ibidem, second paragraph of Answer 15.

332. With regard to the impact of additional information on the conclusions of the safety assessment, **Dr. Healy** concludes that

> The additional molecular characterisation data sought by the lead CA in 2002 were not required to demonstrate the safety of food products derived from MS8, RF3 or MS8xRF3 hybrid lines.

The European Communities submits that, if the sequenced plant DNA regions, adjacent to the transgene inserts, are provided and compared to the corresponding sequences at the site of integration in the isogenic lines, this additional information allows to conclude on the fact that besides the insertion of the intended fragment, no other changes in the DNA sequence have taken place during the transformation process. If this is the case, this is a positive element to conclude on the safety of the GMO.

333. In the following bullet-points on **pages 9 and 10**, **Dr. Healey** gives the rationale for her conclusions. In the **last bullet-point**, she affirms that,

> The most significant elements of the assessment of MS8 and RF3 oilseed rape lines more appropriately focus on the molecular characterisation of the insertion events and the resultant novel gene products, particularly in terms of their potential toxicity and allergenicity. The food products derived from transgenic oilseed rape are likely to contain at most only trace amounts of plant proteins due to processing. Comparative compositional data from the seeds of both transgenic lines and the corresponding isogenic lines therefore provides necessary information to determine unintended effects.

According to the European Communities, risk assessment has to include more than only taking into account the impact caused by the main product from a crop. In other words, also non-intended effects in side products and on the environment as a whole have to be considered. As with regard to the last sentence, comparative sequence data between the transgenic lines and the corresponding isogenic lines is exactly what the lead CA requested. Thus, this paragraph justified this additional request by the lead CA. The European Communities respectfully submits that this conclusion is in contradiction with the reasoning of Dr. Healy.

334. As with regard to the analysis of **Dr. Snape** of **Exhibit EC063/Att.98**, the European Communities notes that the molecular data were incomplete, as sequences at integration loci were not compared to sequences at corresponding sites in wild-type isogenic lines.

335. Finally, in the **conclusion by Dr. Snape**, the European Communities notes that the repeated reference to "MsR8" is not clear and would appreciate clarifications from this expert.

Question 17

Were detection methods commercially available in 2001 sufficient to enable the detection of the transgenic proteins expressed by the plant line hybrid oilseed rape MS8/RF3? Given the information before the Panel, including the SNIF (EC-63/At.109) and the updated environmental risk assessment (EC-63/At.110-140), was additional information regarding a quantitative detection method (EC-63/At.141) necessary to ensure that conclusions of the safety assessment were valid?

General comments

336. Question 17 addresses two issues: whether the detection methods commercially available at the time of the request of the lead CA for additional information regarding a quantitative detection

method sufficient to enable the detection of the transgenic proteins expressed by the plant line hybrid oilseed rape MS8/RF3; and whether this request for information was necessary to ensure that conclusions of the safety assessment were valid. Two Panel's experts answered this question: Dr. Healey and Dr. Nutti. With regard to the first issue, they both conclude that such a method was available, but Dr. Nutti specifies that it had not been validated. On the second issue, they agree that a quantative detection method as required by the lead CA was not necessary for safety conclusions, however Dr. Nutti points out that such a method would have provided relevant information for labelling and the consumer.

337. The European Communities respectfully considers that the question posed by the Panel is vitiated by a misunderstanding. As it is clear from EC-63/At.141, the lead CA points to the need of a quantitative detection method "to facilitate post-marketing control and inspection" and does not refer to the need for such in a safety assessment as suggested by the question. As remarked by Dr. Nutti, the necessity of having a detection method is linked to monitoring and traceability of GMOs after release. For post-marketing control and inspection, as explained by many of the experts, (1) an event-specific method is required and (2) this can only be the case when the method is based upon the presence of foreign DNA and not upon the presence of foreign protein. Therefore, the conclusions of the experts with regard to the necessity of such a method for safety assessment are of no help in understanding the scientific value of the request of the lead CA with regard to post-marketing monitoring and control.

338. The European Communities provides below ***Detailed comments*** with regard to the experts' opinions on the preference for protein- versus DNA-based methods. However, in the context of the lead CA's request, this is not of high relevance.

Detailed comments

339. With regard to the **conclusion of Dr. Healy on the first part of question 17**, the European Communities underlines that it is not possible to conclude on the availability of a commercial kit for PAT detection in 2001. As **Dr. Nutti** rightly points out in its **first paragraph** of her answer to question 17, the method was not validated and, presumably, not commercially available.

340. The European Communities respectfully disagrees with **Dr. Healy** statement in the **penultimate paragraph of page 12** that

> Quantitative PCR methods have been developed for a range of food commodities including soybean and maize products but validation of these methods is not straightforward.

341. In fact, the recently accepted modular validation approach for GMO methodologies perfectly allows to validate quantitative PCR methods, according to internationally accepted, harmonized guidelines[136]. Collaborative trial validation of quantitative PCR methods presently included in the draft international standards has been done in a number of slightly different ways (prEN ISO 21571 Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods). However, essentially the principles are the same: Samples of unknown content (test samples) are compared to samples with assigned concentrations (reference materials). In some cases the DNA has to be extracted from the samples (in which case validation includes validation also of a DNA extraction component), while in other cases

[136] See ISO 5725, 1994; Horwitz W (1995) Protocol for the design, conduct and interpretation of method performance studies. Pure Appl Chem 67, pp.331-343.

the samples distributed consist of pre-extracted DNA (in which case validation includes only the PCR components). A recent paper[137] provides justification for separate validation of DNA extraction and PCR components, - primarily linked with cost efficiency and improved flexibility in the application of methods. The number of methods included in prEN ISO 21571 is currently 13 (all collaboratively validated), while the number of methods included in EN ISO 21572 (Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products - Protein based methods) for comparison is one!

342. Not only does this fact demonstrate the feasibility of validating quantitative PCR methods, it also demonstrates that quantitative protein-based methods of detection are less directly accessible, in contrast to the statements of Dr. Healy. that

> … it is generally recognised that quantitative protein-based methods of detection are more directly accessible.

343. Notably, the protein based methods also have a much more narrow application range than PCR based methods. To the European Communities's knowledge world-wide there is no single quantitative protein-based method that has been validated and considered fit for the purpose. Dr. Nutti seems to agree with this viewpoint when she affirms that: "I can infer that there was no general validation of the method used by the notifier". The European Communities also wishes to underline that accessibility can be interpreted in different ways. First, it can be discussed whether ELISA versus real-time PCR is favourable in different conditions. This largely depends on the tradition and historical perspective concerning these technologies. Second, it can be discussed on the accessibility of the target to be detected: (1) DNA can be readily isolated from most samples; (2) DNA, in contrast to proteins, is much more stable and generally present in different types of matrices and thus enable a more horizontal applicability; (3) PCR is recognized and accepted as the reference method for GMO identification and quantification for regulatory purposes in the EU, because of those advantages; (4) DNA-based methods for GMO quantification, i.e. DNA extraction protocols and quantitative PCR assays can be easily found in the literature and in annexes of the ISO norms for DNA-based GMO detection.[138]

Question 18

Given the information before the Panel, including the SNIF and the updated environmental risk assessment (referenced above), was the information regarding molecular data requested by the CA (EC-63/At.144) necessary to ensure that conclusions of the safety assessment were valid?

General comments

344. This question was answered by two Panel's experts: Dr. Healy and Dr. Snape. Dr. Snape has answered this question together with question 16. His conclusion is, therefore, the same, which is to say that "more information is still required". On the contrary, Dr. Healy concludes her assessment considering that the additional information sought by the CA was not necessary to ensure that that conclusions of the safety assessment were valid. Dr. Healey adds that "given the pace of advancing knowledge in the field of molecular biology and plant biotechnology and the rapidly evolving technical capabilities, for a foreseeable time, there will often be a discrepancy between the scientific data submitted in a dossier for assessment, and the information possible from the most recently developed experimental techniques".

[137] Holst-Jensen A, Berdal, KG (2004) The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds. J AOAC Int 87, pp.927-936.

[138] ISO/FDIS 21571, 2004; ISO/DIS 21569, 2004; ISO/DIS 21570, 2003.

345. The European Communities respectfully disagrees with the conclusion of Dr. Healy and points to the fact that it is the duty of a regulatory authority to continually update its requirements taking new methodologies into account. In particular, the Belgian guidelines on the requirements on molecular information were developed with the help of a panel of scientists specialised in gene technology and experts from the European Association of Bioindustries ("EuropaBio", the association of biotech industries in Europe). The need for the demands for molecular data in these guidelines has been thoroughly discussed in the light of safety assessment and is purely science based. Bio-informatic analysis enables one to check for the presence of novel chimeric open reading frames at the transition between plant and insert DNA or in the region of rearranged DNA fragments. This way, the risk of production of allergens, toxins or other pharmacologically active proteins in the transgenic crop can be assessed. This approach can therefore be a valuable tool for biosafety evaluation as long as other (genomic, proteomic or metabolomic) techniques aimed at the assessment of potential unintended effects of the transformation are not operational. In addition, bio-informatic analysis can also reveal if a transgene has inserted inside a plant ORF. It must be noted that bio-informatic analysis is not only required according to the Belgian Guidelines for molecular characterisation of GM higher plants to be placed on the market, but have also been accepted as a tool in safety assessment by the EuropaBio[139] and the European Food safety Authority.[140] In addition, the guidelines for conducting safety assessment of GM foods that were adopted by the Codex Alimentarius Commission[141] require information sufficient to identify any new substances that may be included in food. DNA sequence information on the junction regions of the transgene is one mechanism to obtain this information.

346. In the specific of this case, the new data provided by the notifier in September 2003 in response to the request from the lead CA was necessary to ensure that conclusions of the safety assessment were valid. The original sequence data describing the integrated inserts were incorrect, and several errors were detected when the transforming plasmids and the integrated inserts were sequenced for verification (cf. Exhibit EC63/At.131 and EC63/At.132). Fortunately, these errors were concluded insignificant with respect to the conclusions of the risk evaluation. On the contrary, a significant unintended rearrangement of an inserted truncated gene cassette in Rf3 was observed and characterised (EC63/At.115). Without a detailed sequence based characterisation of this rearrangement also with respect to its flanking sequences, it would not be possible to predict the potential risk associated with the rearrangement. The nature of the integration sites in the recipient genome was determined through sequence characterisation of the 5-prime and 3-prime flanking sequences of the inserts in Ms8 (EC63/At.114) and Rf3 (EC63/At.115), and these were confirmed to be of host plant origin with a high degree of probability (EC63/At.116-119, EC63/At.133 and EC63/At.134). However, no functional assignments of these sequences were provided in the reply from the notifier. To this date, two of the flanking sequences are believed to represent genes coding for proteins for which it is claimed that "*overexpression causes pleiotropic phenotypic changes*". Pleiotropic phenotypic changes means unpredicted or unexpected changes in the appearance of presumably unrelated or unlinked characters induced by the character under study. Such a statement would therefore undoubtedly attract the attention of risk assessors, if it had been provided by the notifier. It is, however, likely that this particular information was unavailable to the notifier by September 2003. Information that was available without being forwarded until specifically requested concerned the sequence of the flanking sequences (cf. EC63/At.114 and EC63/At.115). The

[139] EuropaBio, 2003. Molecular Characterisation in Guidance document for Safety Assessment of GM Crops.

[140] EFSA, 2004. Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal 99, 1-94.

[141] Codex Alimentarius Commission (2003), Principles for the risk analysis of foods derived from modern biotechnology. Codex Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA plants, CAC/GL 45-2003.

publication of genetic rearrangements and the flanking sequences of GTS 40-3-2 (RoundupReady®) soybeans[142] led to rapid establishment of consensus concerning the relevance of detailed (sequence based) genetic maps of the inserted and flanking sequences. The detection methods provided (EC63/At.136 and EC63/At.137) are not event specific and not quantitative. Furthermore, the specificity of the methods was only assessed against *Brassica napus* samples (EC63/At.138). It is common practice in peer review journals to require considerably wider taxon samples in this kind of specificity test.

347. More ***Detailed comments*** on the information relating to molecular data in this notification to complete the assessment of **Dr. Healy** are provided below. For comments to **Dr. Snape**'s response, the European Communities refers to its comments to answer 16.

Detailed comments

348. The EC chronology includes several exhibits which are relevant for this issue.

349. In particular, EC-63/At.114, which forms an integrated part of the updated risk assessment of Sept 2003, was completed on November 26th 2001, and shows that the flanking sequences of Ms8 were available and known to Aventis CropScience almost two years before making it available to the CA. See page 4 of the exhibit:

> To demonstrate the nature of the flanking sequences of *Brassica napus* elite event Ms8, PCR analysis was performed on a number of templates using different primer-pairs. In a first PCR reaction the identity of the Ms8 template was confirmed using the primer-pairs described in the Ms8 Discriminating PCR protocol. A primer-pair targeting the flanking sequence was used to demonstrate the nature of the flanking sequence. The PCR analysis demonstrated unequivocally that the characterized flanking sequences are present in all tested *Brassica napus* entries and are thus of *Brassica napus* plant origin.

350. Exhibit EC63/At.115 on confidential information on the characterisation of Rf3, which forms an integrated part of the updated risk assessment of September 2003, was completed on November 26th 2001, and shows that the flanking sequences of Rf3 were available and known to Aventis CropScience almost two years before making it available to the CA.See from page 4 of the exhibit:

> To demonstrate the nature of the flanking sequences of *Brassica napus* elite event Rf3, PCR analysis was performed on a number of templates using different primer-pairs. In a first PCR reaction the identity of the Rf3 template was confirmed using the primer-pairs described in the Rf3 Discriminating PCR protocol. A primer-pair targeting the flanking sequence was used to demonstrate the nature of the flanking sequence. The PCR analysis demonstrated unequivocally that the characterized flanking sequences are present in all tested *Brassica napus* entries and are thus of *Brassica napus* plant origin.

351. Genetic rearrangements as a result of the integration event are common.[143] The nature of such rearrangements is not predictable, and each case may therefore deserve examination as part of the risk

[142] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

[143] Qpcrgmofood (2004). Final report of workpackage 3 "Sequence characterisation of transformation events (junction between insert and insertion site in the genome)" QLK1-1999-01301. Available at <http://www.vetinst.no/Qpcrgmofood/Deliverable3.pdf> (last visited on 27 January 2005).

assessment procedure. In Rf3 rearrangement was observed at the 3-prime end, as described in paragraph 4.2 on page 8 of the document:

> The integration of the T-DNA resulted in a large plant DNA duplication: the flanking sequences at the 3-prime end (1275 bp in total) consist of 816 bp duplication of 5-prime flanking sequences ('duplicated 5-prime flanking sequence') and 459 bp ('3-prime flanking sequence'). The identity of the 'duplicated 5-prime flanking sequence' is described in 4.1.).

352. Also Exhibit EC63/At.116, containing confidential alignment with transformation plasmid of Ms8 and which forms an integrated part of the updated risk assessment of September 2003, was completed on October 10th 2001. It shows that the flanking sequences were not derived from the Ms8 transforming plasmid.

353. Exhibit EC63/At.117 on confidential BLAST with flanking sequences of Ms8, which forms an integrated part of the updated risk assessment of September 2003, was completed on September 26th 2001, and shows that no similarity to known sequences was observed, with exception for some similarity to an 87 bp sequence derived from the closely related species *Arabidopsis thaliana*. Apparently, no attempts were made to determine if any function was assigned to the sequence, although this would be permitted in connection with the BLAST search. The similar sequence motif in *Arabidopsis thaliana* is not assigned any function in the NCBI/GenBank database as per this date.

354. Exhibit EC63/At.118 on confidential alignment with transformation plasmid of Rf3, which forms an integrated part of the updated risk assessment of September 2003, was completed on October 10th 2001, and shows that the flanking sequences were not derived from the Rf3 transforming plasmid.

355. Exhibit EC63/At.119 on confidential BLAST with flanking sequences of Rf3, which forms an integrated part of the updated risk assessment of September 2003, was completed on October 8th 2001, and shows that both the 5-prime and 3-prime flanking sequences show similarity to known sequences from the closely related species *Arabidopsis thaliana*. The 5-prime flanking sequence shows similarity to two 97 bp and 33 bp sequences derived from *Arabidopsis thaliana*. Apparently, no attempts were made to determine if any functions were assigned to the sequences, although this would be permitted in connection with the BLAST search. Both the 97 bp and 33 bp sequence motifs in *Arabidopsis thaliana* are assigned function in the NCBI/GenBank database as per January 18th, 2005, cf. **Box 1** and **Box 2**, and both are coding for proteins. Notably, it is stated with the accession in Box 1 in a description of the function of the protein that "*overexpression causes pleiotropic phenotypic changes*". The European Communities has not been able to determine whether these accessions were released by September 2003, although the accession described in **Box 1** was submitted to GenBank on March 11th 2003. The 3-prime flanking sequence shows similarity to a 52 bp sequence derived from *Arabidopsis thaliana*. Apparently, no attempt was made to determine if any function was assigned to the sequence, although this would be permitted in connection with the BLAST search. The 52 bp motif in *Arabidopsis thaliana* is assigned function in the NCBI/GenBank database as per January 18th 2005, cf. **Box 3**, and it is coding for a protein. Notably, the retrieved accession is very similar to the accession shown in **Box 1**, and for both it is believed that "*overexpression causes pleiotropic phenotypic changes*". The European Communities has not been able to determine if the accession described in **Box 3** was released by September 2003, although it was submitted on March 11th, 2003.

Box 1. Sequence retrieved from Blast search on the NCBI server January 18th 2005, applying the 97 bp sequence motif from *Arabidopsis thaliana* as a probe.

```
LOCUS       AY254319                123 bp    mRNA    linear   PLN 04-MAR-2004
DEFINITION  Arabidopsis thaliana DVL3 mRNA, complete cds.
ACCESSION   AY254319
VERSION     AY254319.1  GI:37955425
KEYWORDS    .
SOURCE      Arabidopsis thaliana (thale cress)
  ORGANISM  Arabidopsis thaliana
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots;
            rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
REFERENCE   1  (bases 1 to 123)
  AUTHORS   Wen,J., Lease,K.A. and Walker,J.C.
  TITLE     DVL, a novel class of small polypeptides: overexpression alters
            Arabidopsis development
  JOURNAL   Plant J. 37 (5), 668-677 (2004)
   PUBMED   14871303
REFERENCE   2  (bases 1 to 123)
  AUTHORS   Wen,J., Lease,K.A. and Walker,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-MAR-2003) Biological Sciences, University of
            Missouri, 308 Tucker Hall, Columbia, MO 65211-7400, USA
FEATURES             Location/Qualifiers
     source          1..123
                     /organism="Arabidopsis thaliana"
                     /mol_type="mRNA"
                     /db_xref="taxon:3702"
                     /chromosome="2"
                     /map="BAC F1N21"
     CDS             1..123
                     /note="similar to DVL1; DVL gene family member;
                     overexpression causes pleiotropic phenotypic changes"
                     /codon_start=1
                     /product="DVL3"
                     /protein_id="AAP13818.1"
                     /db_xref="GI:37955426"
                     /translation="MKGTKKKTPCNKKLGGYLKEQKGRLYIIRRCVVMLICWHD"
ORIGIN
        1 atgaaaggta ccaagaagaa gacgccatgc aacaaaaagc ttggaggata cttgaaagag
       61 caaaagggaa ggctttacat catcagaaga tgtgtggtca tgctcatttg ttggcatgac
      121 taa
//
```

Box 2. Sequence retrieved from Blast search on the NCBI server January 18th 2005, applying the 33 bp sequence motif from *Arabidopsis thaliana* as a probe.

```
LOCUS       CNS0AEJL                607 bp    mRNA    linear   HTC 06-FEB-2004
DEFINITION  Arabidopsis thaliana Full-length cDNA Complete sequence from clone
            GSLTSIL6ZB03 of Silique of strain col-0 of Arabidopsis thaliana
            (thale cress).
ACCESSION   BX818257
VERSION     BX818257.1  GI:42475241
KEYWORDS    HTC; GSLT_cDNA.
SOURCE      Arabidopsis thaliana (thale cress)
  ORGANISM  Arabidopsis thaliana
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots;
            rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
REFERENCE   1  (bases 1 to 607)
```

```
  AUTHORS   Castelli,V., Aury,J.M., Jaillon,O., Wincker,P., Clepet,C.,
            Menard,M., Cruaud,C., Quetier,F., Scarpelli,C., Schachter,V.,
            Temple,G., Caboche,M., Weissenbach,J. and Salanoubat,M.
  TITLE     Whole Genome Sequence Comparisons and 'Full-Length' cDNA Sequences:
            A Combined Approach to Evaluate and Improve Arabidopsis Genome
            Annotation
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 607)
  AUTHORS   Genoscope.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-NOV-2003) Genoscope - Centre National de Sequencage:
            BP 191 91006 EVRY cedex - FRANCE (E-mail: seqref@genoscope.cns.fr
            - Web: www.genoscope.cns.fr)
COMMENT     The sequences are based on single pass reads.
            Life Technologies (a division of Invitrogen) members carried out
            full-length librairies construction: Temple G.
            Genoscope members carried out sequencing and annotation: Castelli
            V., Aury J.M., Jaillon O., Wincker P., Menard M., Cruaud C.,
            Schachter V., Weissenbach J., Salanoubat M.
            URGV INRA: Clepet C., Caboche M.
            Annotation is based on the June 2003 version of the Arabidopsis
            genome released by MIPS (Munich Information center for Protein
            Sequences). 5 prime and 3 prime are assembled with Phrap.
            http://www.genoscope.cns.fr/externe/sequences/Banque_Projet_EF/Full
            _length
            http://www.genoscope.cns.fr/cgi-bin/ggb/ggb?source=Arabidopsis.
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                     /strain="Col-0"
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      481 tctaatgtta gtgtatgata tagagtaagg agagaaaaat tataagctta gtttgtatat
      541 gcacacagag agtagttgtt agttgaattt ccttgtctct aattaatcca agtcttcctt
      601 cttgtta
//
```

Box 3. Sequence retrieved from Blast search on the NCBI server January 18th 2005, applying the 52 bp sequence motif from *Arabidopsis thaliana* as a probe.

```
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DEFINITION  Arabidopsis thaliana DVL1 mRNA, complete cds.
ACCESSION   AY254203
VERSION     AY254203.1  GI:37955419
KEYWORDS    .
SOURCE      Arabidopsis thaliana (thale cress)
  ORGANISM  Arabidopsis thaliana
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots;
            rosids; eurosids II; Brassicales; Brassicaceae; Arabidopsis.
REFERENCE   1  (bases 1 to 156)
```

```
  AUTHORS   Wen,J., Lease,K.A. and Walker,J.C.
  TITLE     DVL, a novel class of small polypeptides: overexpression alters
            Arabidopsis development
  JOURNAL   Plant J. 37 (5), 668-677 (2004)
   PUBMED   14871303
REFERENCE   2  (bases 1 to 156)
  AUTHORS   Wen,J., Lease,K.A. and Walker,J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-MAR-2003) Biological Sciences, University of
            Missouri, 308 Tucker Hall, Columbia, MO 65211-7400, USA
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       61 ataagtagaa gattggggaa gtatatgaag gaacaaaagg gaaggattta catcatcaga
      121 agatgtatgg tcatgctcct ttgttcgcat gattga
//
```

356. Exhibit EC63/At.120 on confidential description of Rf3 transgene locus, which forms an integrated part of the updated risk assessment of September 2003, was completed on April 8th 2002, and shows that 5 bp 'filler' has been introduced between the 5-prime flanking sequence and the 5-prime end of the inserted sequence.

357. Exhibit EC-63/At.131 on confidential determination of the inserted sequences in Ms8, which forms an integrated part of the updated risk assessment of September 2003, was completed on August 15th 2001, and includes an alignment of the sequence of the putative transforming plasmid pTHW107 with the sequence of the inserted sequence present in Ms8. This was apparently a re-analysis and verification of earlier data, and proved to demonstrate several minor errors in the earlier data. The errors observed were the following. For the first fragment, covering the 5-prime flanking sequence and the *bar* gene cassette, two insertions of one nucleotide each, one deletion of two nucleotides, and three (one plus two) nucleotide substitutions, were observed. None of these observed differences occurred in coding regions. For the second fragment, covering the 3-prime flanking sequence and the *barnase* gene cassette, three single nucleotide insertions and one deletion of two nucleotides were observed. The deletion was located in the 5-prime end of the PTA29 promoter sequence which regulates the transcription of the *barnase* gene cassette. The reanalysis also revealed that the inserted sequence was identical with the true sequence of the transforming plasmid, i.e. different from the putative plasmid sequence included in the original data submitted by the notifier.

358. Exhibit EC-63/At.132 on confidential determination of the inserted sequences in Rf3, which forms an integrated part of the updated risk assessment of September 2003, was completed on September 11th 2001, and includes an alignment of the sequence of the putative transforming plasmid pTHW118 with the sequence of the inserted sequence present in Rf3. This was apparently a re-analysis and verification of earlier data, and proved to demonstrate several minor errors in the earlier data. The errors were the following. For the first fragment, covering the 5-prime flanking sequence and the *bar* gene cassette, three single nucleotide and one double nucleotides insertions, one double

nucleotides deletion and one single and two double nucleotides substitutions were observed. The double nucleotides insertion was located in the pSsuAra promoter which regulates the transcription of the *bar* gene cassette. For the second fragment, covering the TA29 promoter, no deviations from the putative transforming plasmid sequence were observed. For the third fragment, covering the promoter region of the anter specific TA29 gene a double nucleotide deletion and a single nucleotide insertion was observed. The deletion was located at the 5-prime end of the PTA29 promoter sequence. For the fourth fragment, no deviations from the putative transforming plasmid sequence were observed. For the fifth fragment, covering the 3-prime flanking sequences and the second (inverted) of the two integrated *barstar* gene cassettes, two single nucleotide insertions, one double nucleotide deletion and a single nucleotide substitution was observed. The deletion was located at the 5-prime end of the 3'nos which defines the terminus of the transcription of the second (inverted) *barstar* gene cassette.

359. Exhibit EC-63/At.133 on confidential information analysis of integration sequences Ms8, which forms an integrated part of the updated risk assessment of September 2003, was completed on September 26th 2003, and

> "revealed meaningful sequence similarity of the flanking plant DNA fragments of oilseed rape event Ms8 with published *Brassica oleracea* sequences. None of these *Brassica oleracea* sequence entries in the database are annotated. The intrinsic bioinformatics methods used (Open Reading Frame search; analysis of the first ATG codon context sequences; core promoter and 3'UTR regulatory motifs search) did not produce evidence that a novel transcript might arise at either junction of the oilseed rape Ms8 insert."

360. Exhibit EC-63/At.134 on confidential information analysis of integration sequences Rf3, which forms an integrated part of the updated risk assessment of September 2003, was completed on September 26th 2003, and

> "revealed meaningful sequence similarity of the flanking plant DNA fragments of oilseed rape event Rf3 with published *Brassica oleracea* and *Arabidopsis* thaliana sequences. None of these sequence entries are annotated in the database. The intrinsic bioinformatics methods used (Open Reading Frame search; analysis of the first ATG codon context sequences; core promoter and 3'UTR regulatory motifs search) did not produce evidence that a novel transcript might arise at either junction of the oilseed rape Rf3 insert."

361. Exhibit EC-63/At.136 on confidential Ms8 discriminating method shows that this method is not event specific, it is qualitative and it has only been assessed with DNA extracted from leaf punch or single seed material, i.e. it is not certain whether it would work with low concentrations of GM material.

362. Exhibit EC-63/At.137 on confidential Rf3 discriminating method shows that this method is not event specific, it is qualitative and it has only been assessed with DNA extracted from leaf punch or single seed material, i.e. it is not certain whether it would work with low concentrations of GM material.

363. Exhibit EC-63/At.138 on confidential demonstration of specificity of methods for Ms8 and Rf3 shows that the methods are here only tested on *Brassica napus* samples (event Ms1, Ms8, Rf1, Rf2, Rf3 and wild type). It is not certain whether it would be specific with other samples, e.g. other GM events.

Question 19

Given the information before the Panel, including the SNIF, the updated environmental risk assessment and the clarification provided by the notifier (EC-63/At.147), was the information regarding ecological effects of this product on agricultural systems requested by the lead CA (EC-63/At.149) necessary to ensure that conclusions of the safety assessment were valid?

General comments

364. Three Panel's experts answered this question: Dr. Andow, Dr. Snow and Dr. Squire. They all concur that the information on ecological effects requested by the lead CA was not available in the notification. Dr. Snow considers that "the information on the indirect effects of introducing herbicide-tolerant crops could be considered as necessary because they are still being investigated in the UK". However, she questions "the feasibility of obtaining scientific data on all of the processes listed by the CA prior to deregulation without extensive multi-year studies and underlines the vagueness of the request of the lead CA asking for some unnecessary data". Dr. Squire writes that "it is legitimate to ask what such effects might be since cropping with transgenic oilseed rape (MS8/RF3) and its herbicide has potentially new effects on arable flora (i.e. different from those of other oilseed rape varieties with other herbicides), which may be severely depleted in any case. The need for an appropriate comparator against which the new technology should be judged is noted". And Dr. Andow states that "...although the UK-FSE trials will not be published until later in 2003, everyone involved was aware of the experiments and the possibility that the associated herbicide use would have adverse environmental effects. These issues are not addressed in the response of the notifier. Hence, I conclude that the request by the lead CA for more information on point 9 was necessary to ensure that conclusions of the safety assessment were valid."

365. The European Communities concurs with the overall conclusion of the experts that it was legitimate to ask what such effects could be. One of the principles of an environmental risk assessment is that it should be carried out in a scientifically sound and transparent manner based on available scientific and technical data. For this reason, an environmental risk assessment has to be up-to-date and should cover all the relevant issues even where uncertainty remains. Moreover, if new information on the GMO and its effects on human health or the environment becomes available, the environmental risk assessment may need to be re-addressed in order to 1) determine whether the risk has changed, and/or 2) determine whether it is necessary to amend the risk management accordingly.

366. For its general comments on the persistence of HT products and their potential as "pests", the European Communities refers the Panel to its comments under Question 6 above. In the specifics of this case, it can easily be taken for granted that the large scale application of broad spectrum herbicide in farmland area will cause wide-spread and serious disruption of trophic structures and food webs as the food basis of all species feeding on anything but the crop is eliminated at least temporarily and locally. The severity of this effect will be a function of the area sprayed and the frequency of applications. Such effects were all anticipated at that time and before 1998, however, solid data on GMHT crop production systems were lacking at time that would have allowed to better quantify such effects. The publication of the first conclusions of the FSE trials on 8 October 2003 was a sufficient reason to support the request of the lead CA. One could criticise the way the question has been formulated, the type of information asked, and the level of detail of the questions but fundamentally it was legitimate to ask the notifier to complement its environmental risk assessment with the available data on the ecological impact of the novel weed management regime. In fact, data on safety issues related to combined use of the GM HT crop with the herbicide, including indirect effects, could have been missing from the original environmental risk assessment because of a different interpretation of the regulatory framework by the notifier and the lead CA. According to the lead CA, the indirect effects of herbicide treatments on the farmland biodiversity through the weed population and, more in

general, novel weed management associated with the commercial release of transgenic herbicide-tolerant crops should be considered as a novel agronomic and management technique and should be assessed under any legal framework, and could therefore be addressed under Directive 2001/18/EC.

Detailed comments

367. With regard to the remark of **Dr. Andow** in **paragraphs 19.05** and **19.06**, it is true that the data on epigaeic predators and honeybees was weak at that time and remains so until today. It is questionable whether with some more studies provided the knowledge would have improved greatly. This field was and remains to be quite understudied and highly complex.

368. With regard to the remark of **Dr. Andow** in **paragraph 19.08**, the European Communities notes that the "feeding" study on rabbit was, in fact a single digestibility study. This study only compared the nutritional values of Ms8*Rf3 as a feed with respect to its conventional counterpart through estimation of the digestible energy and the digestibility coefficients and is therefore neither a toxicological study nor a feeding study. A chicken feeding study is mentioned in the environmental risk assessment but the specific report was not included in the application. In fact, the lead CA made a mistake in this statement as no chicken feeding study has been carried out with the event Ms8*Rf3. The chicken feeding studies carried out by BCS included other events.

369. As per comments included in **paragraphs 19.09** and **19.10**, OSR indeed participates in geochemical cycling. Of course, like anything else in nature, also OSR participates in geochemical cycling and there are simple methodologies and experiments that can be conducted to provide at least some initial basic insights.

370. The European Communities also concurs with the assessment of **Dr. Andow** in **paragraph 19.12** of his response, that one 'benefit' of GMHT crops used for promotion of the products is that no-tillage cultivation becomes possible. This is a very significant change in agricultural practice indeed. More information on this is necessary if that is to be evaluated in the approval process.

371. With regard to the comment by Dr. Andow, contained in **paragraph 19.13**, on the length of time it took the lead CA to review the information received and to articulate the questions, the European Communities refer the Panel to its ***General comments*** above.

<u>Trifolium/Monsanto/Danisco Roundup Ready fodder beet (A5/15)</u>
C/DK/97/01 (EC chronology 64)

Question 21

Given the information before the Panel, including the lead CA's positive opinion (EC 64/At. 29-30) and the conclusions of the EC SCP (EC-64/At.83), did the additional risk assessment provided by the notifier (EC-64/At.104-105) in response to requests by the Dutch CA for a theoretical safety assessment address outstanding scientific concerns related to the potential risks associated with this product?

General comments

372. Two Panel's experts have addressed this question: Dr. Andow and Dr. Nutti, and they reach opposite conclusions. According to Dr. Andow, "the additional risk assessment would have addressed

outstanding scientific concern related to the potential risks associated with this product".[144] On the contrary, Dr. Nutti, without much of an explanation on the reasons, concludes that the additional risk assessment provided by the notifier "does not address outstanding scientific concern since they are not related to the potential risks associated with this product", and "was not necessary in the first place".[145] The European Communities agrees with Dr. Andow.

373. The European Communities provides below more detailed comments on the information relating to molecular data in this notification. As with regard to the comment by **Dr. Andow**, contained in **paragraph 21.04**, on the length of time it took the Dutch authorities to review the information received and request additional risk assessment, the European Communities refer the Panel to its general comments above.

Detailed comments

374. The EC chronology includes several exhibits which are relevant for this question, in particular the following ones.

375. Exhibit **EC-64/At.029**, containing a short summary report from the Danish Environmental Protection Agency [EPA] on the dossier, dated 7 October 1997, states

> Southern blot and PCR analysis made on the genetic modified line A5/15 and following generations show that only one gene, cp4-epsps, from the vector is inserted and that the inserted DNA is stable. Subsequently the evaluation was restricted to insertion of the one gene, cp4-epsps, giving tolerance to glyphosate.

376. Exhibit **EC-64/At.083** containing the report from EC Scientific Committee on Plants, dated 23 June 1998, states that

> 2. *Terms of reference*. The Scientific Committee on Plants is asked to consider whether there is any scientific reason to believe that the placing on the market of genetically modified fodder beet tolerant to glyphosate with the purpose to be used as any other fodder beet is likely to cause any adverse effects on human health and the environment.
>
> …
>
> 6.1.3. *Transgenic construct in the genetically modified plant:* Vector pMON17204 was designed to transfer DNA located between the right and left borders. In the A5/15 construct it was determined that only part of the DNA between the borders were transferred. Molecular analysis based on the Southern blot technique showed that the insert contains only the *cp4 epsps* gene. The *uidA*, *gox* and *npt*II genes located between the borders in the vector were not incorporated into line A5/15. By PCR experiments it was stated that the plasmid origins of replication (oriV and oriColE1) were not incorporated into line A5/15. The T-DNA was truncated in the E9 3' element before the 35S promoter and the *uidA* gene resulting in a fully functional *cp4 epsps* gene and no other elements of pMON17204 are inserted into line A5/15. Southern blot analysis showed that there is one copy of T-DNA inserted into line A5/15.

[144] Responses to Questions Submitted by Dr. Andow of 6 January 2005, para. 21.03.
[145] Answers by Dr. Nutti of 7 January 2005, p. 16.

> Stability of the insert was determined in two ways:
>
> (a) Testing of multiple generations of RR hybrids for tolerance suggested that the levels of tolerance are consistent between generations.
>
> (b) Physical stability testing by Southern blot and PCR walking experiments were performed on the original transformation event (T0) and on 5-6 plants from each of the subsequent 3 generations (T1 to T3). It is indicated that no differences in the banding pattern were observed among the generations.
>
> The fact that no meaningful differences between the ranges and mean levels of CP4 EPSPS in A5/15 were observed over 2 years in samples from field trials is consistent with stable insertion and expression of the RR gene over generations.

377. Exhibits EC-64/At.104 and 105, containing a confidential letter from Monsanto to Dutch CA, dated 19 October 1999, where it is stated that:

> During our last discussion in your office on August 4, you have confirmed that the Dutch experts wanted to see new information: 1) either molecular characterization data demonstrating the absence of backbone sequences in the beet genome; 2) or a theoretical safety assessment based on the hypothesis that the entire backbone sequences are present in the beet genome.
>
> We are currently in the process of generating further data to confirm the absence of the backbone sequences, and this information should be available in the following months. In the interim we are providing a risk assessment as requested under point 2 (See attachment).

378. It is not uncommon to conduct a theoretical safety assessment based on a worst case scenario. The lack of available detailed documentation demonstrating experimentally the absence of the backbone (plasmid/vector) sequences precluded a more accurate and reliable safety assessment. In this situation a theoretical assessment could logically be performed on a worst case scenario, i.e. under the assumption that the backbone sequences were present in the GMO. Since such sequences at least theoretically could lead to establishment of chimeric open reading frames (ORFs) encoding proteins or other gene products of unknown function, the theoretical safety assessment addressed outstanding scientific concerns related to potential risks associated with the product.

379. Up until late in the 1990's little attention was paid to detailed molecular characterization of GMOs, primarily because of technological limitations. As technological solutions were made available that would allow for more detailed characterization, i.e. improved PCR, cloning kits and automated sequencing tools, the genetic maps became more detailed. Parallel to this development, the literature grew rapidly on genetic rearrangements and unintended genetic effects associated with transformation. When the CEN/TC 275/WG 11 had its first meeting in Berlin in February 1999, researchers from several European countries were independently already planning projects that would focus on detailed characterization of the inserted and flanking sequences (often referred to as the border sequences of the transformation event), and development of event specific quantitative detection methods. Previous communication with biotechnology companies and competent authorities by some of these researchers had made it clear that the companies were generally unwilling to provide any kind of sequence information. Furthermore, the competent authorities were sometimes either unable to communicate requests for verified insert and border sequence information to the biotechnology companies, or they were sometimes hesitant to do so. In effect, requests were

not always forwarded to the biotech companies, or they were sometimes lacking sufficient precision. However, given the high level of awareness in the biotechnology industry in relation to gene technology, it is very unlikely that the notifier was unaware of the growing demand for such information. The technology required to determine the border sequences was available since the mid-1990's,[146] and that to determine the insert sequences was available several years earlier.

Question 22

Given the information before the Panel, including the conclusions of the SCP's risk assessment, was the information requested by the Italian CA in March 2000 (EC-64/At.116) necessary to ensure that conclusions of the safety assessment were valid?

General comments

380. Two Panel's experts addressed this question: Dr. Andow and Dr. Nutti. Dr. Nutti, without explaining the reasons for her answer, comes to the conclusion that the request of the Italian authority was not necessary, as far as food safety was concerned. Dr. Andow briefly elaborates on each of the aspects and finally considers that only the question on transfer and recombinant in natural conditions was necessary to ensure that conclusions of the safety assessment were valid.

381. The European Communities considers that the Italian CA was justified in requesting this further information in order to have the latest information on these issues and to determine likely frequency of such gene flow in order that risk management strategies could be developed.

Detailed comments

382. The Italian CA had asked for additional information on the efficacy of glyphosate, gene transfer and gene flow to wild relatives. Italy is a major beet seed producing country and thus is concerned to protect the production of high quality seeds by maintaining high levels of seed crop purity and quality.

383. **Efficacy of glyphosate** is an issue because it would be needed to control contamination of beet seed crops with non GM and off-type beets, as well as weeds. However, information on this could also have been gleaned from data on efficacy of glyphosate from other sources (eg pesticide registration).

384. There would also have been concerns about crop safety and that seed yields or quality would not be reduced by applying glyphosate to HT seed crops. Such information was not provided in the application though there were comments on crop (root) yields and performance showing that it is often enhanced compared with conventional herbicides. This was also shown by some experts work[147].

385. On the issue of **gene flow to wild relatives**, the SCP said that cross pollination and gene flow are known to occur between cultivated beets and wild beet types. Different forms of beet are

[146] Liu et al. 1995, TAIL-PCR, The Plant Journal 8(3): pp. 457-463.

[147] MAY, M.J. (2003) Economic consequences for UK farmers of growing GM herbicide tolerant sugar beet. Annals of Applied Biology, 142: 41-48.

interfertile, as shown by the gene flow detected between wild and cultivated beets[148] and by the transfer of useful genes of ssp maritima to new varieties of sugar beet[149].

386. Studies showed that gene introgression occurred[150] potentially creating additional problems for conventional beet seed producers. This is because the introduction of GM beet may result in flow of transgenes to wild beets, where they are incorporated into these wild populations[151]. Subsequent flow of transgenes from wild beet to non-GM beet seed crops could introduce these genes into beet seed and weed beet contaminants of beet seed. This could have important implications for the seed industry.

387. Also, the SCP did not address in its Opinion the management of re-growth of HT beet roots left in the soil after harvest which can flower and cross pollinate with other beets and weed beet. This is especially important in seed production areas which are often close to sugar beet production areas in Italy. Glyphosate herbicide is often used in conventional crop rotations to destroy such volunteer plants regrowing from root residues and conventional beet growers should change their practice as well.

388. It was also important to know the tendancy for bolting (adventitious flowering in the first season) of the beet varieties likely to be transformed particularly as, in the past, fodder beets used to show more tendancy than sugar beet varieties.

Amylogene starch potato
C/SE/96/3501 (EC chronology 67)

Question 25

Given the information before the Panel, including the application (EC-67/At.13-44), and additional information provided by the notifier (EC-67/At.51, 57, 61-63, 75-83, 87, 92-93, 94-95, 101, and 103), was the information regarding molecular characterisation, toxicity, protein analysis, animal feed trials, effects on non-target organisms, bleomycin resistance, and substantial equivalence requested by the Scientific Committee for Food (SCF) (EC-67/At.84-86, 96, 98, 100, 102, 104, 105 and 106), necessary to support a valid safety assessment?

General comments

389. Four Panel's experts have answered this question: Dr. Andow, Dr. Healy, Dr. Nutti, and Dr. Snape. Dr. Nutti considers that "the information required was necessary to support a valid safety assessment. The notifier failed to provide information on several fundamental issues concerning

[148] Santoni S. and Bervillé A., 1992: Evidence for gene exchanges between sugar beet (Beta vulgaris L.) and wild beets: consequences for transgenic sugar beets. Plant Molecular Biology 20: 578-580; Boudry P., Mörchen M., Saumitou-Laprade P., Vernet Ph., Van Dijk H., 1993. The origin and evolution of weed beets: consequences for the breeding and release of herbicide-resistant transgenic sugar beets. Theoretical and Applied Genetics, 87: 471-478; Bartsch D. and Schmidt M., 1997. Influence of sugar beet breeding on populations of Beta vulgaris ssp. maritima in Italy. Journal of Vegetation Science, 8: 81-84.

[149] Debock T.S.M., 1986. The genus Beta: domestication, taxonomy and interspecific hybridization for plant breeding. Acta Horticulturae 182: 335-343.

[150] Bartsch D., Cuguen J., Biancardi E., SweetJ. 2003. Environmental implications of gene flow from sugar beet to wild beet – current status and future research needs. Environ. Biosafety Res. 2: 123-127.

[151] Arnaud J.F., Viard F., Delescluse M., Cuguen J., 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in Beta vulgaris (Chenopodiaceae): consequences for the release of genetically modified crops species with weedy lineages. Proc. Roy. Soc., London, B270, 1565-1571.

safety such as substantial equivalence, toxicity, protein analysis and animal feeding trials." Dr. Snape reinforces this point with regard to molecular characterisation by concluding that "more information is still required". Similarly Dr. Andow, who only commented on the "non-target organism" part of the question, states that "a request for additional information was scientifically justified in 1999 and remains justifiable even today". On the contrary, Dr. Healy concludes that: "The information requested after [20 July 2000] (and much of the information requested prior to this date) is unlikely to influence the conclusions of the safety assessment."

390. The European Communities shares the conclusions reached by Dr. Andow, Dr. Nutti, and Dr. Snape. Detailed comments on the reply by Dr. Healy are provided below.

Detailed comments

391. With regard to **molecular characterisation**, the European Communities concurs with the analysis of Dr. Snape. The questions of the SCP address problems of elements in the flanking region ("confusing and inconclusive PCR analyses to demonstrate absence of vector sequences flanking the border regions") as well as the antibiotic specificity of the nptII gene[152]. The question of the SCP to the molecular characterisation of the backbone of the plasmid as well as the question of the quality of the southern blot (overlapping probes, analysis of nos gene region, controls) are according to the present state of the art of a characterisation. The question to the detailed level of molecular characterisation including an analysis of potential ORFs and expression of genes is in accordance with the present state of the art for a molecular characterisation of a transformation event as defined by Codex, FAO/WHO consultations and scientific literature,[153] which requires a precise and detailed characterisation including sequencing in flanking regions outside the boarders (where it is not agreed how many bps outside the boarder). Although this was probably not so clearly defined at the time of the assessment by the SCP, the SCP reacted to upcoming evidences about possible unintended effects of insertions asking for a detailed molecular characterisation of the product. The requests of the SCP are confirmed in their validity by the standards presently accepted.

392. As far as **toxicity and feeding trial** are concerned, the identification of an ORF with transcription and unclear translation was a consequence of the inefficient molecular characterisation at this time and could have been of safety relevance. The argument of Dr. Healy on compositional equivalence does not add to safety as a risk assessment. Looking for substantial equivalence at this time would not likely have detected hazards from such an ORF event. In fact, the requested feeding trials might have not added substantially to the safety assessment but have been the best possible reaction to an unsolved problem and the available tools at this time.

393. On the issue of **protein analysis**, Dr. Healy agrees with the requests for additional information formulated before July 2000. On the contrary, on the SCP requests of 20 July 2000 (EC-67/At.100), 1 February 2001 (EC-67/At.102), 14 March 2001 (EC-67/At.104), 3 April 2001 (EC-67/At.105) and 8 November 2001 (EC-67/At.106), she concludes that "sufficient information is available" and that "**further analysis including Western blotting requiring ORF4** specific antibodies is not justified".

[152] See Exhibit EC-67/At.84.

[153] Codex Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA plants, CAC/GL 45-2003. FAO/WHO expert consultation, 2003. D.A. ANDOW, D.A. SOMERS, N. AMUGUNE, F. ARAGAO,K. GHOSH, S. GUDU, E. MAGIRI, W.J. MOAR AND E. OSIR, Transgene Locus Structure and Expression of Bt Maize, Environ. Risk. Chap 04 in: CAB International 2004. Environmental Risk Assessment of Genetically Modified Organisms: Vol. 1. A Case Study of Bt Maize in Kenya (eds A. Hilbeck and D. Andow).

394. The European Communities considers the request for detailed sequence comparison as very important because it shows, inter alia, the still existing different point of view on the assessment of allergenicity. The Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA plants explains that

> … sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate.

395. A report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2001, concluded that:

> 5.6.1. Level of expression
>
> Highly allergenic proteins are often expressed at relatively high levels. However, allergens can sensitize susceptible individuals at less than milligram levels, possibly at less than microgram levels (Sorva et al., 1994; Jarvinen et al., 1999). ….Thus, level of expression cannot yet be incorporated into the assessment of the allergenicity of genetically modified foods.
>
> In achieving the objective of conferring a specific target trait (intended effect) to the host organism by the insertion of DNA sequences, additional traits could, theoretically, be acquired or existing traits lost or enhanced (unintended effects). Unintended effects may be due to factors such as random insertion events, which might result in disruption of existing genes and modification of protein expression.
>
> 6.1. Sequence Homology as Derived from Allergen Databases
>
> The commonly used protein databases (PIR, SwissProt and TrEMBL) contain the amino acid sequences of most allergens for which this information is known. However, these databasesare currently not fully up-to-date…… Structural similarity with known allergens may still be important if significant amino acid identity is found, but it is below 35 %. In this case significant cross-reactivity is unlikely…..Since identity of 6 contiguous amino acids has an appreciable risk of occurring by chance, verification of potential crossreactivity is warranted when criterion (1) is negative, but criterion (2) is positive. In this situation suitable antibodies (from human or animal source) have to be tested to substantiate the potential for crossreactivity.
>
> Conclusion of the Consultation:
>
> The original decision tree from the FAO/WHO 2000 Consultation served as a basis for this consultation. The Consultation concurred that this decision tree be modified as a consequence of more recent research and which is reflected in the FAO/WHO

> 2001 decision tree. When the expressed protein is derived from a source with no known allergenicity, the FAO/WHO 2001 decision tree proposes that the initial investigation would also be analysis of sequence homology to known allergens from food and environmental sources. If positive matches are found with known allergens, then the protein is considered likely allergenic. If no significant sequence homology is identified, then targeted serum screening is conducted with serum samples that contain high levels of IgE antibodies with a specificity that is broadly related to the gene source. If the targeted serum screening is positive, then the protein is considered likely allergenic. If the targeted serum screening is negative, then pepsin resistance of the expressed protein and the immunogenicity of the expressed protein in suitable animal models.

396. The latest FAO/WHO consultation on GM foods of 2003 agrees that

> It has been recognized that there is no single parameter that can predict the allergenic potential of a substance. A strategy to assess allergenicity of biotechnology products has been formulated (FAO/WHO, 2001; Codex Alimentarius Commission, 2003), which relies on the parameters: source of the gene, sequence homology, serum testing of patients known to be allergenic to the source organism or to sources distantly related, pepsin resistance, the prevalence of the trait and assessment using animal models"

397. The Consultation recommended that additional efforts should be directed to the further development and validation of models and that there is a need to improve the accessibility and interconnectivity of existing databases or to establish a centralized database on allergenic linear and conformational epitopes and tools for screening transgenes for allergenic potential. Even the present strategy based on a sequence prediction model established by FAO/WHO was contradicted by important groups.[154]

398. These facts demonstrate that there is no agreed detailed, standardized common system for safety assessment of allergencity and even nowadays many experts feel that assessment schedules need to be improved. Therefore the conclusion of Dr. Healy that "sufficient information is available to support the conclusions of the safety assessment" without additional information as required by the SCP, cannot be accepted. Already at the time of the debate, the SCP adhered to scientific uncertainties in the assessment of a potential allergenicity of GM foods which are even valid and more concrete nowadays. The requirement of tests including antibodies/sera is widely accepted in unclear situations nowadays. The assessment of the consequences of a new ORF with a potential protein with no history in risk assessment or food safety is certainly a case where additional testing is required according to the Codex Guideline.

399. With regard to Dr. Healy's discussion of the SCP request for "in vitro data on the survival of the APH(3')II protein from leaf tissue in the presence of rumen micro-organisms (and of ORF4, if expressed)" *(*EC-67/At.85), the European Communities respectfully submits that Dr. Healy is overstepping her mandate to analyse molecular characterisation by discussing the risks associated with antibiotic resistance genes. The international understanding these issues has evolved substantially over the last years (see the European Communities' comments under questions 1 and 2 above) and there is now wide international agreement that horizontal transfer of genes, especially antibiotic resistance genes, from GM plants or products derived from such plants to micro-organisms,

[154] Stadler and Stadler, Allergenicity prediction by protein sequence, FASEB, J 2003, 17, 9, 1141-3. Difficulties in the concept are summarised in Jank and Haslberger, Improved evaluation of potential allergens in GM food, Trends Biotechnol., 2003, 21, 6, 249-250.

especially in the gut, is unlikely, but not impossible. In consequence of this state of the art, an appropriate risk assessment, based on a case by case approach, in respect to a possible transfer of genes from a GMO to micro-organisms of the GI tract or in other relevant ecosystems is necessary and has to include aspects of the gene such as the resistance and the consequence of a possible transfer of the resistance. Dr. Healy agreed with the relevance of this evaluation to determine whether the potential ORF4 polypeptide would confer bleomycin resistance (on bleomycin, see more below).

400. Similarly, the European Communities respectfully submits that Dr. Healy has overstepped her mandate when assessing the SCP request for data on the safety of modified crops to **non-target organisms** (EC-67/At.84).

401. With regard to **bleomycin resistance**, the dossier submitted contained strong indication that (a) an unknown protein may have been synthesised in the potato tubers and (b) that this protein may have encoded resistance against the antibiotic bleomycin which is a chemotherapeutic used in cancer treatment. The potential risk was due to the use of a transformation vector, pBin19, which is broadly used in plant transformation. Therefore, this dossier could have had consequences beyond this specific file. In this light, the SCP has scrutinised well all data, has had several meetings with the applicant and has concluded only when all no further doubts existed. Consequently, the process might have been lengthy but the delays where all based on safety considerations.

402. On the issue of **substantial equivalence**, the European Communities notes that, in principle the expert agrees with the need of this kind of assessment. This discussion needs also to be seen in the light of developments and changes of concepts at this time. The concept – that a comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessments of GMOs – was a commonly used basis for the development of both, food safety and the environmental risk assessment.[155] This concept was elaborated by FAO, WHO and OECD in the early 1990s and referred to as substantial equivalence for the assessment of GM foods. But in 2000, a FAO/WHO consultation acknowledged that this concept had attracted criticism from the perception that it was the end-point of a safety assessment rather than the starting point.[156] Furthermore, evidences gathered about considerable variation in constituents in GMOs in response to environmental conditions.[157] The Consultation concluded that a consideration of compositional changes is not the sole basis for determination of safety and that safety can only be determined when the results of all aspects under comparison, ad not merely comparisons of key constituents are integrated. More recently the concept has evolved to a Comparative Safety Assessment for GMO foods.[158]

403. Finally, with regard to **unintended changes** in GM crops detected by metabolic profiling, the European Communities would like to refer the Panel to a study using four independent potato

[155] World Health Organization. 1991. Strategies for assessing the safety of foods produced by biotechnology. Report of a Joint FAO/WHO Consultation. World Health Organization, Geneva. Switzerland.

[156] FAO/WHO 2000; Safety aspects of genetically modified foods of plant origin, a joint FAO/WHO consultation on foods derived from biotechnology, Geneva, Switzerland 29 May - 2 June 2000. Millstone et al.,1999; Millstone E, Brunner E, Mayer S. Beyond 'substantial equivalence' Nature. 1999 Oct 7;401(6753):525-6. Schenkelaars, 2002: Schenkelaars P. Rethinking substantial equivalence. Nat Biotechnol. 2002 Feb;20(2):119.

[157] Novak,W.K.; Haslberger,A.G., Substantial equivalence of antinutrients and inherent plant toxins in genetically modified novel foods., Food Chem.Toxicol., 2000, 38, 6, 473-483.

[158] Kok EJ, Kuiper HA., Comparative safety assessment for biotech crops., Trends Biotechnol. 2003 Oct;21(10):439-44.

genotypes characterized by modifications in sucrose metabolism[159]. This research has general implications for food/feed safety and for environment (target and non-target organisms could be affected in unpredictable ways) but also supports case-by-case assessments, as each GM event could be uniquely different to the parental line or isogenic (non-GM line). Nine lines for altered sucrose metabolism were transformed, and 88 metabolites analyzed. Of the 88, the majority exhibited significant changes in levels in 1 or more of the 9 transgenic events v. conventional potatoes. In addition, 9 novel metabolites (not present in conventional potatoes) were discovered in some or all of the transgenic lines. This seems to be a striking indication of how genetic engineering can cause widespread alterations in cellular metabolism. The study also has an estimate of 90,000 to 200,000 for the number of different molecules in the combined plant kingdoms; while Arabadopsis has roughly 5,000. If 5,000 is typical for a species, and if the proportion of unmeasured potato compounds whose level is altered is anything like the "over half" of the 88 this study measured, one could be talking significant alterations in the expression levels of hundreds to thousands of genes, plus dozens or hundreds of novel compounds. Granted that most of these changes would likely not have any adverse impacts, it is still a large pool of alterations that, it seems to me, would be likely to harbour a few nasty ones.

Monsanto Roundup Ready oilseed rape (GT73)
C/NL/98/11 (EC chronology 70)

Question 27

Given the information before the Panel, including the notification, was the information regarding feed safety aspects of this product requested by the Netherlands (EC-70/At.8 and 13) necessary to ensure that conclusions of the safety assessment were valid?

General comments

404. Only one Panel's expert, Dr. Nutti, answered this question. Her conclusion is that the studies requested by the Dutch authorities were not necessary to ensure the product's safety assessment. The European Communities respectfully disagrees because, as it will be shown in details below, very serious reserves on the interpretation of the results and on the conclusions of the rat experiments can be formulated. This especially as Dr. Nutti addresses only some aspects of the safety assessment. She does not at all address the aspects of unintended effects based on sincere problems in the molecular characterisation, which are at the centre of concern of the CA. The analysis of potential unintended effects is a central element in the guidelines for a safety assessment in CODEX.

Detailed comments

405. With regard to the **feed safety** aspects, the Dutch authority requested a whole report to evaluate composition (Exhibit EC-70/At.08) as well as the experimental results of semi-chronic rat and trout studies and an assessment of the relevance of the performed studies in experimental animals to predict the safety of the product in target animals such as ruminants, pigs and poultry (EC-70/At.13). This request for additional experiments is particularly justified because these target species are known to be particularly sensitive for the detection of anti-nutritional factors (ANFs) such as goitrogenic effects of GT73, already suspected on rats.

[159] Roessner U., Luedemann A., Brust D., Fiehn O., Linke T., Willmitzer L., Fernie A.R., 2001. Metabolic Profiling Allows Comprehensive Phenotyping of Genetically or Environmentally Modified Plant Systems, in The Plant Cell, Vol. 13, 11–29.

406. It is clear from Exhibit EC-70/At.02 that very unsatisfactory experimental designs have been used in rats experiments. The results on feeding studies were not accurately presented. The first 6-week feeding study revealed an increase in the absolute liver weight (+ 5 / 20 %) in male rats fed with GT 73. The second 4-week experiment confirmed the observations of the previous experiment. An increase in the relative liver weight (+ 12-16 % in males for a level of 15 % of toasted meal) has been observed. In addition, a mixture of seeds of different GT 73 lines has been acknowledged in the second experiment: "Inadvertent commingling of seeds …",[160] *sic*. Furthermore, the experiment was conducted apparently with only one group of animals fed GT 73, without a control group.

407. What is also to be noted when considering the answer provided to this question is that the assessment of toxicology, such as requiring feeding studies, needs to be supported by important aspects of **molecular characterisation**. Dr. Nutti does not at all consider the aspects of unintended effects based on problems in the molecular characterisation, nor could she relied on an answer to question 26, but – nonetheless – she comments on toxicological relevant questions. This discrepancy (especially in the possible understanding of the basis of her toxicological comments) deserves further examination.

408. As seen above, the question of the Dutch CA addresses sincere lacks in the safety assessment of the product, especially significant flaws in the molecular characterisation. If the molecular characterisation of a product is not adequately provided or if the characterisation indicates potential hazards (such as rearrangements in flanking regions, unclear ORFs or not-conclusive results of sequence analysis for toxicological assessment) at least toxicological testing using other methods, such as feeding studies, are required to exclude hazards from unintended effects which can not be clarified by the molecular characterisation. However, one might/should argue that results from non observed effects in toxicological relevant feeding studies cannot serve as a substitute of necessary conclusive results from a molecular characterisation.

409. Whereas the need for analysing potential unintended effects and an adequate molecular characterisation was already discussed at the time of the CA request, recently important scientific organisation confirmed and detailed this procedure. In a FIFRA SAP meeting of the US-EPA, the expert panel agreed on the relation between molecular characterisation and toxicology testing:

> Screening for unintended effects is encouraged. Such a screening could use profiling methods for unintended effects, e.g. expression microarrays or metabolomic studies (e.g., as discussed in SAFOTEST). However, as these tests are still under development, toxicology studies in rodents, looking for effects of unintended effects, although unlikely, could be appropriate. While the targeted safety assessment system in place today has apparently been effective in risk characterization, it cannot eliminate the chance that unintended or unexpected consequences of plant breeding will escape detection. It would be extremely useful to have comparative data regarding the frequency and magnitude of unintended effects associated with conventional plant breeding with or without the use of genetic engineering techniques upon which a quantitative risk assessment could be based[161].

[160] See page 73 of the ACNFP Review of Glyphosate-tolerant Oilseed Rape, annexed to the Application contained in Exhibit EC-70/At.02.

[161] Minutes of the FIFRA Scientific Advisory Panel Meeting Held June 8-10, 2004: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management.

410. As with regard to Codex, Dr. Nutti argues that

> … according to the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (Page 4, paragraph 53), some foods may require additional testing; regarding animal feeding studies, extra studies may be warranted if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. Although the product was concluded to be substantially equivalent …

411. This summary of the Codex Guidelines is not comprehensive and is misleading. The cited paragraph 53 needs to be analysed together with paragraphs 11-13 and 17, which states that:

> … Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can therefore be extremely difficult. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.
>
> Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the safety assessment of foods derived from food plants, including recombinant-DNA plants. This has been addressed by the development of a multidisciplinary approach for assessing safety which takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, using the concept of *substantial equivalence.*
>
> The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart[2]. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.
>
> …
>
> The safety assessment of foods derived from recombinant-DNA plants involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information are necessary to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health.

412. The misleading conclusion that a risk assessment according to the substantial equivalent *per se* can establish safety was corrected explicitly in 2000 when a FAO/WHO consultation acknowledged that this concept had attracted criticism because it was the end-point of a safety

assessment rather than the starting point[162]. The Consultation concluded that a consideration of compositional changes is not the sole basis for determination of safety and that safety can only be determined when the results of all aspects under comparison, ad not merely comparisons of key constituents are integrated. More recently the concept has evolved to a Comparative Safety Assessment for GMO foods.[163]

413. The latest FAO/WHO expert consultation on GM animals, which updated the entire field (where FAO/WHO expert consultations are usually the basis for Codex recommendations) concluded that

> An extensive molecular characterization of the inserted genetic material construct will generally be required, both before and after the insertional event. The molecular characterization should furthermore comprise an analysis of the copy number and a sequence analysis of the flanking regions of the place of insertion in order to identify any unintended effects. It is recommended that the approach for the molecular characterization should be further standardized to include the flanking regions.[164]

414. As the Dutch CA realised that fundamental problems were present in the molecular characterisation, it requested supplementary studies to investigate potential unintended effects as requested in the Codex guidelines for GM plants. The argument that the substantial equivalent did not see any significant changes cannot at all mean that in this case no further testing is necessary, even in the absence of conclusive information from molecular characterisation. The safety assessment carried out according to the substantial equivalent does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart (Codex). Therefore, the requests of the Dutch CA fully adhered to Codex Guidelines.

Question 28

Given the information before the Panel, including the notification and the SNIF (EC-70/At.49-53), was the additional information provided in Monsanto's submission to the Commission (EC-70/At.84-97) necessary to ensure that conclusions of the safety assessment were valid?

General comments

415. Two Panel's experts have provided answers to this questions: Dr. Andow and Dr. Nutti. Dr. Andow considers that questions related to monitoring that refer to specific parts of the risk assessment are justifiable, including Italy's question related to the AMPA metabolite that is formed from the glyphosate (Roundup) herbicide, whilst he perceives the requirements for the monitoring procedures (e.g. Sweden, with regard to human and animal health) as too strict, given that preferably

[162] FAO/WHO 2000; Safety aspects of genetically modified foods of plant origin, a joint FAO/WHO consultation on foods derived from biotechnology, Geneva, Switzerland 29 May - 2 June 2000; Canadian Royal Society: Canadian Royal Society, Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada, an Expert Panel Report on the Future of Food Biotechnology prepared by The Royal Society of Canada at the request of Health Canada, Canadian Food Inspection Agency and Environment Canada, 2001; Millstone et al.,1999; Millstone E, Brunner E, Mayer S. Beyond 'substantial equivalence' Nature. 1999 Oct 7;401(6753):525-6; Schenkelaars, 2002: Schenkelaars P. Rethinking substantial equivalence. Nat Biotechnol. 2002 Feb;20(2):119.

[163] Kok EJ, Kuiper HA., Comparative safety assessment for biotech crops., Trends Biotechnol. 2003 Oct;21(10):439-44.

[164] FAO/WHO Consultation, 2003.

"worst cases" should be monitored for. Dr. Nutti, on the contrary, regards the additionally requested animal experiments as not necessary given the previously provided information and its (positive) evaluation by the EFSA GMO Panel. More ***Detailed comments*** on the experts' replies are provided below.

Detailed comments

416. The European Communities notes that question 28 pertains to additional information provided in Monsanto's submission, whereas replies of the Panel's experts pertain to the validity of some (but not all) of the questions that were addressed by Monsanto.

417. Thus, for example, with regard to Dr. Andow's comments on the strictness of the monitoring pertaining to, among others, procedures requested by Sweden on 28 February 2003, for monitoring for adverse effects to animals (consuming feed) and human health (*cf.* EC-70/At.67-transl, pp. 3-4), the European Communities notes that Sweden had only reiterated, albeit in different but similar wording, the items mentioned in the monitoring plan submitted by Monsanto itself on 13 December 2002[165]. Therefore, the Swedish request posed no new requirements on the applicant. It is also to be noted that the pertinent monitoring plan was updated with more details of the animal feed and human health surveillance by the applicant in July 2003 (EC-70/At. 92, pp. 19-26).

418. In addition, Dr. Nutti's notes that additional animal experiments have been requested. However, this request is not explicitly mentioned or addressed by the applicant in its reply to the Member States comments (EC-70/At.85). One exhibit reviews toxicity studies with rats, which had, however, previously been provided to the rapporteur Member State and therefore did not represent truly new additional information (EC-70/At. 90).

419. With regard to additional information not considered by the Panel experts, Monsanto has provided a certain quantity of additional information consisting of both replies and appendices.

420. Additional information pertaining to **bioinformatic studies** of open reading frames (ORFs, i.e. part of potential new genes) in the DNA flanking the insert created by the genetic modification were requested by the United Kingdom (EC-70/At.85, p. 10, additional information in EC-70/At.88 and 94-96 (MSL18493). These bioinformatics studies of ORFs are in line with Codex guideline,[166] as well as its Annex' paragraphs 8-11, regarding the presence of potential fusion proteins and the toxicity and allergenicity of newly expressed proteins. The additional information was therefore necessary to ensure that conclusions of the safety assessment were valid.

421. With regard to transgenic protein used for toxicity studies, a study showing the **equivalence of the recombinant GOX** protein expressed in GM canola GT73 to that expressed in GM bacteria (*Escherichia coli*) and purified for the purpose of safety testing was requested by Spain (EC-70/At.85, p. 11, additional information in EC-70/At.89 (MSL12968). In addition, the applicability of studies with bacterially produced GOX and EPSPS was doubted by Austria. The provided information is relevant for the safety assessment of a "new substance" in a GM plant, as explained by Codex guidelines, paragraph 40:

[165] EC-70/At.64, pp. 96-97: "Additional information to complement oilseed rape notification C/NL/98/11, as requested by the entering into force of the Directive 2001/18/EC", Annex VII, "Monitoring plan", point 2.

[166] Codex Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA plants, CAC/GL 45-2003, paragraphs 31D and 38.

> This may require the isolation of the new substance from the recombinant DNA-plant or the production of the substance from an alternative source, in which case, the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the recombinant-DNA plant.[167]

The additional information was therefore necessary to ensure that conclusions of the safety assessment were valid.

422. With regard to **compositional analysis of glucosinolates** (toxic), additional information pertaining to the differences observed between GT73 canola and control canola was requested by Austria (EC-70/At.85, p. 13; additional information provided in EC-70/At.90). The comparative safety assessment, also termed the principle of "substantial equivalence",[168] focuses on differences between the GM crop and its conventional counterpart with a history of safe use. Therefore, the statistically significant differences in glucosinolate levels should be considered as to whether they would trigger a further safety assessment. The additional information, providing compositional data from later periods than previously, has an important role in support of the applicant's arguments that the glucosinolate levels are well within safety limits and that their elevation in GM canola GT73 was of a transient nature. The additional information was therefore necessary to ensure that conclusions of the safety assessment were valid.

423. In addition, Monsanto has also provided replies without separate appendices that would have contained original studies or full reports. These replies were provided in response to questions supplementary to those mentioned above. These questions pertained to compositional analysis, herbicide residues, and potential allergenicity.

424. For example, with regard to **compositional analysis**, Member States had sought clarifications about statistical analysis, herbicides used in the experiments, size of samples taken for analysis, potential cross-contamination, and comparability of Canadian with European conditions. With regard to phenotypic and agronomic characteristics, there was a question about the differences observed in one location. These are details that are important in the identification and interpretation of differences observed during the comparative safety assessment approach of "substantial equivalence"[169]. The Member States' questions were therefore relevant to ensure that conclusions of the safety assessment were valid.

425. Some of the Member States' questions with regard to **allergenicity** pertained specifically to **identicalness of transgenic products (GOX, and from other ORFs)** to known allergens and celiac-disease-related proteins. The identicalness at stake consisted of 6- or 7- amino acid identities that had been documented previously by the applicant, and that, in one case, had been identified in a peer-reviewed article as part of an antiserum-binding segment of an allergen.[170] The Annex to the Codex guideline,[171] paragraph 8, recommends that:

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[167] Ibidem, paragraph 40.
[168] Ibidem, paragraph 13.
[169] Ibidem, paragraph 13.
[170] Kleter, G.A., Peijnenburg, A.A.C.M. (2002) Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE - binding linear epitopes of allergens. BMC Structural Biology 2: 8. Available at <http://www.biomedcentral.com/1472-6807/2/8> (last visited on 28 January 2005).
[171] Codex Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA plants, CAC/GL 45-2003, page 40.

> Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for identifying sequences that may represent linear epitopes. The size of the contiguous amino acid segment should be based on a scientifically justified rationale in order to minimize the potential for false positive or false negative results[N].

And in the footnote to this, it reads as follows:

> It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segments in searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

In the light of these recommendations by Codex, it was legitimate for the Member States to ask questions about the 6- and 7- amino acid segment identities. In its reply to these questions, the applicant provided arguments for his choice for an 8-amino acid threshold, *i.e.* its "scientifically justified rationale" as recommended by Codex. The Member States' questions were therefore relevant to ensure that conclusions of the safety assessment were valid.

Pioneer/Dow AgroSciences Bt corn Cry1F (1507)
C/NL/00/10 (EC chronology 74)

Question 32

Given the information before the Panel, including the original notification (EC-74/At.1-15), was the information regarding molecular characterization of this product requested by the lead CA (EC-74/At.17 and 33) necessary to ensure that conclusions of the safety assessment were valid?

General comments

426. Only Dr. Nutti wrote three lines on this question. There she affirmed that "a lot of information was provided", however she felt "not capable of judging if such material was enough to ensure that conclusions of the safety assessment were valid".

427. The molecular characterization provided by the notifier was based on Southern blot analysis and assumptions about the specific sequence of the transforming sequence and the insert. This is not sufficient given the technological development in the 1990's and the accumulated literature on unintended genetic rearrangements etc. that may accompany presumably successful transformations of plants by the time the notification was submitted. The information regarding molecular characterization of this product requested by the lead CA was, therefore, necessary to ensure that conclusions of the safety assessment were valid. The European Communities will provide below some detailed comments on the information requested by the lead CA on molecular characterization.

Detailed comments

428. Table 4 of exhibit **EC-74/At.005** summarizes the observed fragments during Southern analyses of the DNA insert of 1507 maize and may be compared with the predicted fragments listed in Table 3 of exhibit EC-74/At.005. There are several fragments listed in Table 4 that were not predicted in Table 3, and that could not be referred to similar fragments observed in negative control genomic DNA (see footnotes to Table 4). These extra bands may represent genetic rearrangements or duplicate copies of insert elements. The Southern blots presented in Figures 4-7 of exhibit

EC-74/At.005 were not reproduced in sufficient quality to be evaluated. The data provided cannot be said to demonstrate the absence of additional inserted sequences. If such sequences were present, they would deserve to be characterised in more detail to determine whether they are likely to pose a risk to human or animal health or the environment.

429. Question 3 of exhibit **EC-74/At.017** requires the nucleotide sequences of both the vector and the integrated insert, i.e. the sequence that is actually present in the GMO (that may differ from that in the vector). And questions 5-7 of exhibit EC-74/At.017 appear to say that the notifier was required to provide a sequence based characterisation of the DNA sequence(s) that is actually inserted in the GMO. Furthermore, the detection method must be event specific to meet the requirement to confirm the unique molecular identity of the maize line. All other types of detection method will fail to meet the requirement.[172]

430. Question 3 of exhibit **EC-74/At.017**, as presented in exhibit **EC-74/At.019**, and the information provided in Figures 1, 2, and 3 of exhibit EC-74/At.019 appear to give details on the intended sequence of the vector but not of the integrated insert as it is present in the GMO, and possibly also not verified through sequencing of the vector. This is similar to the situation discussed in relation to question 18 (EC chronology 63, exhibits 63/131, 63/132 and possibly also 63/115 above).

431. The answers to questions 5 and 6 of exhibit **EC-74/At.017**, provided in exhibit **EC-74/At.019**, clearly demonstrate that the characterization was based on Southern blot analysis and not supported by DNA sequencing studies. This also means that a range of rearrangements and substitutions and/or insertions and deletions of short sequence motifs (e.g. single or double nucleotides) were unlikely to be detected (see comment under this exhibit question 3, above).

432. Based on the answer to question 3 of exhibit **EC-74/At.033**, provided in exhibit **EC-74/At.054**, the European Communities believes that the inserted sequence in the 1507 maize should be verified by sequencing of the maize, not just by assuming that the sequence of the vector is unaltered. There are several reports of such rearrangements, including a well known and frequently cited paper published a year before this reply,[173] suggesting that a full characterisation may be justified.

433. Although Southern blot analysis with probes of the size applied here (cf. exhibit **EC-74/At.054**, reply to question 4 of exhibit **EC-74/At.033**) is unlikely to detect short fragments that may have been integrated in other parts of the genome, the lead CA according to exhibit EC-74/At.002 seem to conclude that this is not a problem. In this context, Table 4 of exhibit **EC-74/At.005** should be noted, where several unpredicted bands were observed that have not been explained. As commented above, it cannot be concluded that the absence of unintended insertions is demonstrated (exhibit EC-74/At.005).

434. Finally, the detection method seems to target a sequence motif that is not covering the junction between the flanking sequence of the recipient genome and the inserted sequence, but instead target a sequence resulting from a rearrangement of inserted sequences (cf. exhibit **EC-74/At.057**). This sequence motif is likely to maintain its status as unique to 1507 maize also if the same vector is

[172] See e.g. Miraglia, M., Berdal, K.G., Brera, C., Corbisier, P., Holst-Jensen, A., Kok, E., Marvin, H.J.P., Schimmel, H., Rentsch, J., van Rie, J.P. & Zagon, J. (2004; alphabetical order). Detection and traceability of genetically modified organisms in the food production chain. Food Chem. Toxicol. 42: 1157-1180.

[173] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

used to transform new maize or other plants in the future. The sequence is furthermore a single copy sequence in the haploid genome that carries the genetic modification, and as such could be fit for the purpose of DNA based quantitation as well. The detection method is qualitative, not quantitative, and the limit of detection is reported to be 0.1 % on kernel count basis (cf. exhibit **EC-74/At.058**). The length of the amplification product is slightly longer than other typical event specific targets used in real-time PCR assays, and this may potentially limit its applicability for analyses of processed materials in comparison to methods that target shorter sequences. However, no specific requirements concerning the length of amplicons have been raised in connection with the requests from the lead CA.

Question 34

Given the information before the Panel, including the original notification and additional information provided by Pioneer (EC-74/At. 18-13, 35-50, and 53-59), was the information regarding feed safety, detection methods and reference materials requested by the lead CA (EC-74/At.52 and 60) necessary to ensure that conclusions of the safety assessment were valid?

General comments

435. Only Dr. Nutti addresses this question asserting that the requests from the Dutch CA were correct and necessary. Only with regard to the request on detection method (EC-74/At.60), she considers herself not apt for judging.

436. On this regard, the European Communities would like to note that the data relating to the detection has not yet been validated according to international standards (see http://crl-gmo.jrc.it) but has only been tested in the laboratory where it has been developed. This is a completion of the first step of the process, i.e. the development and initial characterisation of the method. The second step is the evaluation of the data by an independent laboratory (under the current legislation EC legislation, this is the Community Reference Laboratory) and the evaluation of the data in the light of the "minimum performance criteria for carrying out a validation study". In the case of Event TC1507, the full validation is currently still on going and therefore, as of today there is no detection method that can be considered as "fit for the purpose".

437. Furthermore, as for question 17, the European Communities wishes to point out that the need for a detection method has been requested in the context of post-marketing surveillance and not in the context of safety assessment.

438. This said, the request contained in exhibit EC-74/At.060 is very clear and detailed with respect to the type of information sought and the scientific basis for requesting the information. Thus, although the need for a detection method is usually considered to be more relevant to consumer policy than to safety, the availability of a reliable detection method constitutes also a precautionary measure. In fact, in the very unlikely event that an authorised GMO is found to be harmful to human health, the availability of a reliable detection method may facilitate efficient tracing and withdrawal of the GMO and its derived products. Without reference materials, no detection method can be considered truly reliable. The request for a detection method and reference material is therefore necessary also from a safety perspective.

Question 35

In your view can EC field trials, in France, Italy, and Chile, provide compositional data on maize kernels that is relevant to evaluating cultivation areas exporting maize to the EC?

General comments

439. Two Panel's experts, Dr. Andow and Dr. Nutti, replied to this question. Their conclusions diverge substantially. Dr. Nutti states that the compositional data on France, Italy, and Chile were supplementary to other previously provided data (which, in fact, they were not) and that therefore, they would not have been necessary. In so doing, she does not really address the question of the Panel. Dr. Andow, on the contrary, clearly concludes that "maize from France, Italy, and Chile would not provide compositional data on maize kernels that is relevant to evaluating cultivation areas exporting maize to the EC".

440. The European Communities concurs with Dr. Andow's above conclusion and will provide below some detailed comments on replies from both experts.

Detailed comments

441. In **paragraph 35.07**, **Dr. Andow** discusses the point that no explicit scientific rationale has been provided for the lead CA's request mentioned above. Whereas the lead CA's request is not mentioned in question 35, it is in line with the Codex guideline, paragraph 45, pertaining to compositional analysis of key components, which reads as follows:

> The location of field trials should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature.[174]

Therefore, while no explicit rationale is provided in the lead CA's request, this is in line with Codex recommendations.

442. Indeed, Dr. Nutti correctly notes that the compositional data should be in line with Codex recommendations, citing paragraph 45 of these guidelines with regard to the choice of field trial location being representative for the various environmental conditions for crop cultivation. However, Dr. Nutti assumes that the compositional data from France, Italy, and Chile has been provided as a supplement to previous data that would comply with Codex guidelines. Perhaps the answer by the applicant (EC-74/At.36, p.5), recapitulating what has previously been provided, might have created this confusion. However, the data from France, Italy, and Chile were the only compositional data provided by the applicant at that time (summarized on pp. 11-26 of EC74/At.7). Based upon the Codex paragraph cited by Dr. Nutti, and given that the application concerned import of maize 1507, the question whether France, Italy, and Chile are relevant for areas exporting maize to the EU would therefore still be relevant. And, as discussed by Dr. Andow, as these countries are not among the major exporters of maize in the world, the answer to the question should be negative.

[174] Codex Guideline for the Conduct of Food Safety Assessment of Foods derived from Recombinant-DNA plants, CAC/GL 45-2003.

Pioneer/Dow AgroSciences Bt corn Cry1F (1507)
C/ES/01/01 (EC chronology 75)

Question 36

Given the information before the Panel, including (EC-75/At.1), was the information regarding molecular characterization, toxicity, and environmental impact of this product requested by the National Biosafety Committee of Spain (NBC) (EC-75/At.5) necessary to ensure that conclusions of the safety assessment were valid?

(a) Given that Pioneer had notified this product under the regulation concerning the deliberate release of this plant (cultivation), were concerns raised by the lead CA on allergenicity and toxicity of the proteins expressed by the inserted gene sequences relevant to the risk assessment of this product?

General comments

443. Both Dr. Nutti and Dr. Andow have provided answers to this question in their respective fields of expertise. Their replies overlap on toxicity issues, on which they take widely differing positions. Dr. Nutti's comments point to a factual misunderstanding dismissed. Dr. Andow's analysis to the greatest extent confirms the lead CA's approach in this instance. Furthermore, the European Communities notes that no expert has commented on the request for additional molecular characterisation and will, therefore, offer a few brief comments itself.

Detailed comments

444. Dr. Nutti's reply on question 36 points to a misunderstanding on the lead CA's side. When stating that "the protein used in the toxicology and allergenicity tests was obtained in a heterologous system and not in other maize" Dr. Nutti bases herself on the data contained in EC 75 At. 1. These data are actually from a later date (February 14th, 2002) than the requests in EC 75 At.5 (November 28th, 2001). In fact, these are the data that the applicant provided in response to the lead CA's request in At. 5.

445. In any event, specifically in the context of food safety the requested information with regard to the source of the transgenic protein is relevant for the safety assessment of a "new substance" in a GM plant, as explained by the Codex guideline CAC/GL 45-2003:

> "This may require the isolation of the new substance from the recombinant DNA-plant or the production of the substance from an alternative source, in which case, the material should be shown to be biochemically, structurally, and functionally equivalent to that produced in the recombinant-DNA plant."[175]

446. Dr. Nutti's reply on question 36a which she links to the lead CA's question on degradation of proteins (question 6 of At. 5) seems to be based on a misunderstanding regarding the chronological order of data submitted as above, and the data discussed in the answer may not have been known to the lead CA at the time of the request. In addition, the lead CA's questions on toxicity and

[175] Codex Alimentarius, 2003. Codex Principles and Guidelines on Foods Derived from Biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, Rome. ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf, {Codex alimentarius, 2003; par. 40}

allergenicity were relevant, since the application for cultivation of maize 1507 also included animal feed purposes.

447. In any event, Dr. Andow provides a thorough explanation on why data on degradation of protein is necessary (Dr. Andow's reply on question 36, para. 36.06). Specifically in the context of food safety, this is confirmed by the recommendations by Codex in its guideline for the safety assessment of foods from plants derived through modern biotechnology:

> In the case of proteins, the assessment of potential toxicity should focus on …… stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems."[176]
>
> The absolute exposure to the newly expressed protein and the effects of relevant processing will contribute toward an overall conclusion about the potential for human risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing which would be applied and its effects on the presence of the protein in the final product.[177]

448. Dr. Andow addresses the environmental issues in great detail. He concludes that most of the requests of the Spanish CA were valid to ensure the conclusions of the safety assessment were relevant to Spain and rigorously conducted. Indeed, toxicity and environmental impact data on other species (e.g. regionally appropriate non target insects, including other non-domesticated herbivores) and regional environments (local growing regions in Spain) would be needed to accurately determine toxicity and environmental impacts to local Spanish fauna of Bt corn Cry1F and its degradation products (i.e. resulting from ingestion by herbivores and decomposition in the soil of plant material and root exudates). Even for target pest species from different countries or regions, sensitivities to expressed Bt toxins vary widely. Hence it can be reasonably expected that the same (species-specific and even population-specific variability in sensitivity to Bt toxins) will apply to local non target species that could be affected by this Bt toxin e.g. local butterflies of conservation concern or of heritage value. There is thus support for the argument that there still was scientific uncertainty about impacts of Bt crops on regionally-appropriate target pest populations and non-target species. Therefore there were scientifically justified environmental concerns (i.e. case-by-case and potentially member state specific) and also unanswered scientific questions over the reliability of the 'high dose and refugia' strategy to work if the particular Bt crop event has not been tested regionally. The point of these specific tests are to ensure that a) the regionally selected species of target pests are susceptible to the expressed Bt toxin AND that b) regionally selected non-target species are not susceptible to the expressed Bt toxin or metabolites, if exposure is considered likely. [178]

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[176] Codex guideline CAC/GL 45-2003, para. 38.

[177] Codex guideline CAC/GL 45-2003, para. 16.

[178] Publications on the issue of target pest and non-target lepidopterans differing in their sensitivity to Bt toxins include Felke, Lorenz & Langenbruch (2002). J. Appl. Entomol. 126: 320-325; Wolt, Peterson, Bystrak & Meade (2003). Environ. Entomol. 32(2) 237- 246; Dutton, Romeis & Bigler (2003). BioControl 27: 441-447; Fitt et al., 2004 Report for International Cotton Advisory Committee, pp 1-64; Fitt 2003; Proc. 3rd World Cotton Research Conf, Cape Town, pp 371-381; Bernal, Griset & Gillogly (2002). J. Entomol. Sci. 37: 27-40. Publications showing species differences in Bt toxin sensitivities for lepidopteran, non-pest species which could have regional- or country-specific conservation or heritage value. i.e. non-target herbivores (butterflies) that could be affected by Bt crops include: Wraight, Zangerl, Carroll & Berembaum (2001). Proc. Natl. Acad. Sci. USA 97: 7770-7773; Zangerl, McKenna, Wraight, Carroll, Ficarello, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 113-118; Sears, Hellmich, Stanley-Horn, Oberhauser, Pleasants, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 119317-42; Jesse & Obrycki (2000) Oecologia 125: 241-248; Jesse & Obrycki (2002). J.

449. Finally, as the issue of molecular characterisation has not been addressed by any expert, the European Communities would like to offer the following brief comments:

450. The European Communities would refer to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 et suiv). As regards the present case, the National Biosafety Committee of Spain (NBC, EC-75/At.5) concluded that the issue of a possible open reading frame (ORF) was not sufficiently addressed, and requested more information on this, along with a verified sequence characterisation of the insert.

451. It should be noted that the dossier is dated July 6th 2001, i.e. several months after the publication of Windels et al. (2001).[179] The applicant, therefore, must have been familiar with the issue of unintended rearrangements and the unintended presence extra fragments. The original dossier did not include essential data for the molecular characterization, such as a verified sequence characterisation of the insert nor flanking sequences or a PCR method for detection of the GMO. The CA's request for additional information was, therefore, necessary to ensure that conclusions of the safety assessment were valid.

Monsanto Roundup Ready corn (NK603)
C/ES/00/01 (EC chronology 76)

Question 37

Given the information before the Panel, including the notification (EC-76/At.1-2 and 27), was further information regarding molecular characterisation, nutritional analysis, and environmental impact requested by the lead CA (EC-76/At.6) necessary to ensure that conclusions of the safety assessment were valid?

General comments

452. Dr. Nutti, Dr. Andow and Dr. Squire have provided answers to this question in their respective fields of expertise. Dr. Nutti's reply seems to be based on an erroneous assumption due perhaps to a misreading of the Spanish text. While disagreeing with some of the requests made by the lead CA, Dr. Andow confirms the necessity of requesting information on gene escape/spillage. In that, he is supported by Dr. Squire who adds further arguments on the relevance of such data for the case at hand. Furthermore, the European Communities notes that no expert has provided comment on the request for additional information on molecular characterisation and will, therefore, offer some brief comments itself.

Detailed comments

453. Dr. Nutti's comment relates to toxicity and nutritional studies in the context of determining substantial equivalence. Her statements acknowledge the importance of the animal studies that have been carried out by the applicant on chicken broilers and rats. The point she makes is that no additional studies beyond a broiler feeding study and a subchronic rat toxicity study are needed: "Whatever studies were further requested by the lead CA in EC-76/At.6, such studies were not necessary." Thus, she seems to assume that the lead CA had asked for additional studies, which would

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Kansas Entomol. Soc. 75: 55-58; Jesse & Obrycki (2003) Agric. Environ. 97: 225-233; Hellmich, siegfried, Sears, stanley-Horn, Daniels, et al., (2001). Proc. Natl. Acad. Sci. USA 98: 11925-30.

[179] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

expand on the previous experiments with broilers and rats. However, the lead CA had not asked for additional studies, but had merely repeated a request for submission of the reports of the chicken broiler and rat studies (in Spanish):

> se *reitera* la petición de remisión de los estudios de alimentación realizados con pollos y los estudios de toxicidad de la proteína a dosis repetidas, tan pronto estén disponibles," (EC76, At. 6, p. 2, par. 8, emphasis added).

454. These reports, which are the ones referred to as necessary by Dr. Nutti herself, were not included in the original application. Following the above renewed request, they were submitted by the applicant in September 2001 (Annexes 4 and 5 of EC76, At. 8_SCI, summary on pp. 20-31, title pages on pp. 39-40).

455. Dr. Nutti, therefore, apart from the erroneous assumption she makes, is in complete agreement with the lead CA in this instance.

456. Dr. Andow is rather critical of the need for certain data, on the basis of a request found in a previous attachment (At5) which, as regards environmental assessment and management plan, he believes would only be needed in the context of cultivation, but not, as is the case here, where the product in question is destined for import only. Two points should be made on this: First, it seems that all three experts discuss different background documents and requests. Second, it may be safe to assume, as does Dr. Andow, that the lead CA was anticipating future cultivation of the product in light of an impending application for cultivation. In light of this, even if one were to argue, that requests relating to herbicide use, non target organisms etc. were outside the scope of the present application, the request for information considered by Dr. Andow would have been relevant if the CA had reasons to believe that the imported grains could have escaped unintentionally and be maintained into the environment, or more likely, that they wanted to better prepare the assessment of a forthcoming application on deliberate release. That CA request does not state at any point that it is "suspensive", and it may therefore simply be a normal cooperative exchange of information between applicants and CA to prepare the ground ffor future or imminent applications.

457. In any event, Dr. Andow suggests that accidental spillage should be the focus of the information provided by the applicant: "Some information is necessary to consider how gene escape can occur either during processing, storage or transport, but detailed information is not necessary." Dr. Squire's reply supports and reinforces this view for the particular case at hand (Spain): "On the more specific question of whether GMHT maize persists (accidental germination), it is justified to query the original statement since emergence has been recorded in some southern European areas."

458. Finally, as regards the request for additional information on molecular characterisation, on which no expert has commented, the European Communities would like to offer the following brief comments:

459. The European Communities would refer to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 et suiv). In the present case, it should be noted that the notification is dated January 2nd 2001. The applicant, therefore, was already familiar with the contents of Windels et al. (2001)[180] and the issues of molecular characterisation of inserts, flanking sequences and possible rearrangements and extra fragments. According to the SNIF

[180] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

(EC-76/At.2) the notification did not include verified sequence data for the full insert, only for the flanking sequences and the 5' and 3' ends of the insert. The Spanish CA's request pertains to these data ("la secuencia genética completa introducida, incluidas las secuencias flanqueantes"), and was, therefore, necessary to complete the risk analysis, and in particular to enable the development of appropriate detection methods. The applicant partially provided the data on September 5th 2001 (EC-76/At.7-8), (but not the verified sequence data for the complete insert).

Question 38

Given the information before the Panel, including the notification and letter from Monsanto providing additional information (EC-76/At.7-9), was additional information necessary regarding molecular composition and environmental impacts associated with accidental germination requested by the lead CA (EC-76/7-9 and 10) necessary to ensure that conclusions of the safety assessment were valid?

General comments

460. Dr. Andow and Dr. Squire both comment on this question in addressing the issue of environmental impact of accidental dissemination or germination. This issue was already addressed by both experts in question 37, where both confirmed the necessity of a request for additional information on this. The present comments add to the debate on the specificity of such a request. Although perhaps generally more critical than Dr. Squire on the question of specificity, Dr. Andow supports the lead CA's request as "necessary to ensure that conclusions of the safety assessment are valid."

461. Furthermore, the European Communities notes that no expert has provided comment on the request for additional information on molecular characterisation. The European Communities would refer to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following).

462. On October 10th 2001 (EC-76/At.10) the request of the CA was for the application of PCR to detect small fragments, and for the application of RT-PCR to detect possible transcripts of a possible unintended open reading frame (ORF). The notifier provides more data concerning the possible ORF in EC-76/At.12. These data clearly indicate that the use of RT-PCR improved the sensitivity of the molecular characterisation in comparison to the Northern blot data provided in the notification. A previously undetected transcript was detected and characterised with RT-PCR, although this apparently did not derive from the possible ORF. Therefore, although the notifier concludes that the conclusion in relation to safety provided in the notification is not altered, one can conclude that the lead CAs request for additional information at this point was necessary to ensure that conclusions of the safety assessment were valid.

Question 39

Given the information before the Panel, including the notification and additional letter from Monsanto providing additional information (referenced above and EC-76/At.11-12), was additional information regarding molecular characterization and toxicology requested by the lead CA (EC-76/At.14) necessary to ensure that conclusions of the safety assessment were valid?

General comments

463. Dr. Nutti and Dr. Squire have commented on parts of this question in their respective fields of expertise. Dr. Nutti addresses the issue of safety margins in feeding studies. As she bases herself on

a document which she cannot have seen (as it has not been submitted to the Panel) her reply must be dismissed. Dr. Squire restates his general reply to questions 37 through 39 with which the European Communities is generally in agreement.

Detailed comments

464. Dr. Nutti refers to the lead CA's request for clarification on the titles of tables 2 and 3 on p. 24 of Appendix 2 ("una aclaración sobre los títulos de las tablas 2 y 3, pág. 24 del Apéndice 2), concluding that that request was not necessary. However, the appendix that the lead CA is referring to is not part of the documents submitted to the Panel. EC 76 Att. 12 SCI only contains the title page of the appendix. It is therefore not known to which details the request of the lead CA pertains. Dr. Nutti's conclusion that the request was not necessary, thus, has no foundation.

465. In general, safety margins for animal feeding, *i.e.* the ratio between the minimum level where adverse effects occur and the actual intake, are important in assessing the risks associated with the intake of a potentially noxious compound. Preferably the safety margin is way above 1, in other words: the intake of a noxious compound should be way below the minimum dose at which it would exert adverse effects. This information was therefore relevant to risk assessors in the interpretation of toxicity data and is addressed, for example, by Codex guideline[181], paragraph 35:

> The safety assessment should take into account the chemical nature and function of the newly expressed substance and identify the concentration of the substance in the edible parts of the recombinant-DNA plant, including variations and mean values. Current dietary exposure and possible effects on population sub-groups should also be considered

466. In addition, paragraph 10 of the Codex guideline also addresses safety margins, as follows:

> It is therefore relatively straightforward to feed such compounds to animals at a range of doses some several orders of magnitude greater than the expected human exposure levels, in order to identify any potential adverse health effects of importance to humans. In this way, it is possible, in most cases, to estimate levels of exposure at which adverse effects are not observed and to set safe intake levels by the application of appropriate safety factors.[182]

467. The same reasoning as for human food derived from GM crops (Codex) applies to animal feed.

468. Furthermore, as identified in EC 76 Att. 27, there were detected changes (two nucleotides) in the CP4EPSPS, and CP4EPSPS L214 P genes, which consequently coded for two new proteins which had not been assessed before. As a consequence, even if it is judged a cautious approach, the CA request proved fully justified *a posteriori*, in light of the subsequent information provided by the applicant.

[181] Codex Alimentarius, 2003. Codex Principles and Guidelines on Foods Derived from Biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, Rome. ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf

[182] Codex guideline CAC/GL 45-2003, para. 10

Question 39bis

Given the information before the Panel, including the notification and additional letter from Monsanto providing additional information (previously referenced and EC-76/At.11-12), was additional information regarding allergenicity studies and PCR tests requested by Austria (EC-76/At.44) necessary or useful to ensure that conclusions of the safety assessment were valid?

General comments

469. Dr. Nutti simply concludes that the request was not necessary without providing any explanation. Her reply, thus, is unfounded and must be dismissed. In making their request, in fact, the Austrian authorities had followed the recommendations of the latest FAO/WHO Expert Consultation on that issue at the time. The request was therefore relevant and necessary.

Detailed comments

470. The request for additional studies on allergenicity came in addition to the simulated stomach degradation studies and computer-aided comparisons with allergens that had already been provided by the applicant. This request had been made by Austria and addressed two issues (EC76, At. 44_transl):

> A request for additional screening of the *transgenic proteins* for binding to sera from allergy patients, and
>
> Another request for testing potential changes in the intrinsic allergenicity of maize caused by the genetic modification, using *whole plant extracts* for these studies

471. The request for this information was dated March 21st, 2003 (EC76, At. 44). At that moment, an FAO/WHO Expert Consultation on the Allergenicity of Genetically Modified Foods had been concluded.[183] This consultation provided input for the establishment of the pertinent Annex on allergenicity testing in the Codex guideline for safety assessment of foods derived from GM crops,[184] which was published on July 9th, 2003.

472. The FAO/WHO Expert Consultation devised a decision tree approach for the testing of potential allergenicity of GM foods, in which serum screening was one of the steps that had to be followed. Serum screening could be either specific (if the foreign gene was from an allergenic source, and therefore sera directed against this allergen should be used) or targeted (if the gene was from a source unknown to be allergenic, and therefore sera against broadly related allergens should be used). Within this approach, a targeted serum screening should have been carried out with the transgenic EPSPS proteins from maize NK603. Austria's question for the serum binding tests was therefore relevant at that time.

473. In addition, potential alterations of intrinsic allergens (which were already present in the GM organism before it became modified) should be considered during the comparative safety assessment (also called "substantial equivalence"). This is because the genetic modification might unintendedly

[183] FAO/WHO, 2001. Joint FAO/WHO expert consultation on foods derived from biotechnology - allergenicity of genetically modified foods - Rome, 22 - 25 January 2001. Food and Agriculture Organisation of the United Nations, Rome.
http://www.who.int/foodsafety/publications/biotech/en/ec_jan2001.pdf

[184] Codex Alimentarius, 2003. Codex Principles and Guidelines on Foods Derived from Biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, Rome. Available at ftp://ftp.fao.org/codex/standard/en/CodexTextsBiotechFoods.pdf

have changed this intrinsic allergenicity of the plant. Intrinsic allergens are mentioned, for example, in the Codex guideline section dealing with compositional analysis of key components, as footnote 5 to paragraph 44:

> "Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens."

474. The experiments proposed by Austria for testing the allergenicity of whole plant extracts included binding tests with sera against a range of plants, as well as sensibilization studies in animals. Both types of tests are in accordance with the decision tree approach recommended by the FAO/WHO Expert Consultation {FAO/WHO, 2001} and are also mentioned in the Codex guidelines as methods and tools that may be considered as scientific knowledge and technology evolves.[185] Austria's question for these tests for altered intrinsic allergenicity was therefore relevant at that time.

475. In EC76, At. 44, Austria also requested availability of the necessary data and material for an analytical method, including the necessary PCR primer sequences, and the reference material. This request appears fully justified and necessary, as no detection method, specific for that transformation event, had been made available at that time by the applicant.

Monsanto Roundup Ready corn (GA 21)
C/ES/98/01 (EC chronology 78), C/GB/97/M3/2 (EC chronology 85)

Question 40

Given the information before the Panel, including the application (EC-85/At.25-26), questions by Denmark (EC-85/At.32) and responses to these questions (EC-85/At.41), was the additional information requested by Denmark (EC-85/At.42) necessary to ensure that conclusions of the safety assessment were valid?

General comments

476. Dr. Nutti only replies to one aspect of the Danish request for additional information, namely the safety of the GM crop for animals, not, however, to the safety of the herbicide applied to maize GA21. Her reply is based generally on a non-comprehensive approach to safety based on compositional analysis and animal toxicity tests with purified proteins (see also the comments on broader risk assessment in the section on general and methodological issues, as well as comments on Q111 to 113) and more specifically on a limited and misleading citation of the Codex Guidelines.

Detailed comments

477. The Danish request comprises two aspects, namely the safety of the GM crop for animals, and the safety of the herbicide used on the GM crop (*i.e.* feeding of *herbicide-treated* GM maize to animals and measurement of transfer of herbicides to edible animal products, in this case milk of dairy cattle). Dr. Nutti only addresses the first, but not the second aspect.

478. As regards the first aspect, Dr. Nutti states that "[a]ccording to the Codex Alimentarius Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA

[185] Codex Alimentarius, 2003; Annex on "Assessment of Potential Allergenicity, para. 17

Plants [...], additional animal testing will be required if the composition is not comparable to conventional foods, which is not the case of Monsanto's event corn GA 21."

479. Several points need to be made. First, Dr. Nutti's general approach to safety based on compositional analysis and animal toxicity tests with purified proteins is non-comprehensive–as explained in the comments on broader risk assessment in the section on general and methodological issues, as well as comments on Q111 to 113. In fact, the very paragraph Dr. Nutti is quoting (i.e. paragraph 53 of the Codex Guidelines) also links the issue of insufficient data on other aspects (*e.g.* molecular characterisation) to the requirements of animal studies, a link which Dr. Nutti herself does not make. Thus paragraph 53 recommends that

> If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.

480. Based on this recommendation and the considerations provided below, it would still be relevant to ask for additional animal feeding studies.

481. Second, it should be pointed out that the Codex Guidelines concern food safety, but not feed safety. Thus, they do not apply directly to the issue at hand.

482. Third, contrary to what Dr. Nutti states, the Codex Guidelines, if applies by analogy, would support a request for additional animal testing.

483. Cattle and pigs belong to the target animals of feed products derived from maize, such as maize gluten. These animals therefore constitute "consumer subgroups" with a different physiology than that of the broiler chicken previously used by the applicant for nutritional testing. The Codex guidelines, when considering human food applications of GM crops (and not animal feed), address the issue of different physiologies of subgroups. The following sentence, for example, has been extracted from paragraph 49 of the Codex guideline section dealing with nutritional modifications:

> Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems.[186]

484. Whereas this paragraph deals with human subpopulations, it can be envisioned that the same applies also to animals with different physiologies in the case of GM crops used for animal feed purposes.

485. In addition, the genetically modified protein introduced by the applicant, the mutant maize EPSPS protein, was different from the bacterial CP4 EPSPS protein previously introduced into other GM crops. This GM crop would therefore be the first crop with this particular modification to be marketed within the EU. The concept of "familiarity" would therefore not be applicable to maize GA21.

486. Furthermore, an OECD document reviewing animal feeding studies, which was published in 2003, recommends nutritional testing with certain animals in case it is indicated that unintended

[186] Codex guideline CAC/GL 45-2003, para 49.

effects might have occurred.[187] This includes, for example, the fast-growing broiler chicken, which are considered to be most sensitive to alterations in nutritional value. Broilers had already been used by the applicant when the Danish CA made its request. The OECD document also mentions the use of other species, such as lactating dairy cattle, of which the milk production can serve as a substitute for growth rate.

487. Taken all these considerations (different physiology, non applicability of "familiarity", and usefulness of animal models) together, contrary to Dr. Nutti's assertions the data requested by the Danish authorities concerning additional animal feeding studies for no treated event maize would have provided relevant information.

488. As regards the second aspect, namely the safety of the herbicide applied to maize GA21, Dr. Nutti does not address it at all. The European Communities offers the following brief comments:

489. With this second issue, the Danish authorities address potential risks for human health: Herbicide residues may remain in products of animals fed with herbicide treated GM crop (e.g. milk).

490. The Codex Guidelines consider this issue in paragraph 54 (Potential accumulation of substances significant to human health):

> Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g., procedures for assessing the human safety of chemicals) should be applied.[188]

491. The questions are therefore relevant from the point of view of safety of the GM crop and any pesticides that might accumulate in it.

Question 40bis

Given the information before the Panel, including the first whole food study and agronomic performance tests (attached, cover letter provided in EC 78/85/At. 19), was a second animal whole food study requested by Denmark, Austria and Italy (EC-78/85 /At. 67, 40 and 72) necessary or useful to identify potential adverse effects that had not been previously identified?

General comments

492. Similar to her reply on question 40, Dr. Nutti's reply is based on a non-comprehensive approach to safety based on compositional analysis and animal toxicity tests with purified proteins (see also comments on broader risk assessment in the section on general and methodological issues, as well as comments on Q111 to 113) as well as limited reference to the Codex Guidelines. The reply, therefore, should be dismissed.

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[187] OECD (2003) Considerations for the Safety Assessment of Animal Feedstuffs derived from Genetically Modified Plants. Series on the Safety of Novel Foods and Feeds, No. 9. Organization for Economic Cooperation and Development, Paris, 46 pp. Available at http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/env-jm-mono(2003)10

[188] Codex guideline CAC/GL 45-2003; para. 54.

Detailed comments

493. Dr. Nutti relies on paragraph 53 of the Codex Guidelines when stating that "my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the biovailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food, and this was not the case of maize GA21."

494. This conclusion is overlooking important issues in at least two respects. First, as set out in the comments on broader risk assessment in the section on general and methodological issues, as well as in the comments on Q111 to 113, Dr. Nutti's approach to compositional analysis is generally non-comprehensive. Indeed, the very paragraph, Dr. Nutti is quoting (i.e. paragraph 53 of the Codex Guidelines) also links the issue of of insufficient data on other aspects (*e.g.* molecular characterisation) to the requirements of animal feeding studies, a link which Dr. Nutti herself does not make. Thus paragraph 53 recommends that

> If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.

495. Based on this recommendation and the considerations provided below, it would still be relevant to ask for additional animal feeding studies.

496. Second, as pointed already, Dr. Nutti cites the Codex guideline for *foods* derived from GM crops, whereas the application at stake pertains to animal *feed purposes* (and incidental human consumption). That said, if one is to draw elements from these guidelines by analogy, the following consideration apply:

497. The target animals of feed products derived from maize, such as maize gluten, include, for example, cattle, pigs, and poultry. These animals therefore constitute "consumer subgroups", which may have a different physiology than that of the broiler chicken previously used by the applicant for nutritional testing. The Codex guidelines (in consider human food applications of GM crops and not animal feed), address the issue of different physiologies of subgroups. The following sentence, for example, has been extracted from paragraph 49 of the Codex guideline section dealing with nutritional modifications:

> Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems."[189]

498. Whereas this paragraph deals with human subpopulations, it can be envisioned that the same applies also to animals with different physiologies in the case of GM crops used for animal feed purposes.

499. In addition, an OECD document reviewing animal feeding studies, which appeared after the request had been done, recommends nutritional testing with animals showing high production, which are considered to be most sensitive to alterations in nutritional value of the GM crops.[190] This

[189] Codex guideline CAC/GL 45-2003, para. 49.

[190] OECD (2003) Considerations for the Safety Assessment of Animal Feedstuffs derived from Genetically Modified Plants. Series on the Safety of Novel Foods and Feeds, No. 9. Organization for Economic Cooperation and Development, Paris, 46 pp..

includes, for example, the rapidly-growing broiler chicken, which has already been used by the applicant. Also lactating dairy cattle, of which milk production and characteristics can be measured, may serve as a model, in which case milk production would substitute for growth rate.

500. Furthermore, the genetically modified protein introduced by the applicant, the mutant maize EPSPS protein, was different from the bacterial CP4 EPSPS protein previously introduced into other GM crops. This GM crop would therefore be the first crop with this particular modification to be marketed within the EU. The concept of "familiarity" would therefore not be applicable to maize GA21.

501. In light of these considerations, contrary to Dr. Nutti's conclusion, the data requested by the three Member States' CAs would have provided relevant information to assure the safety of maize GA21.

Question 41

Given the information before the Panel, including the notification (EC-87/At.1), additional letters from Monsanto providing additional information (EC-87/At.3, 7, CBI part in 8, and 14) and the conclusion of the Commission on Genetic Modification that this product did not present environmental risks (EC-87/At.8-9), was additional information regarding data on the composition of high-oleic soybeans and the alteration in the protein profile of this product requested by the lead CA (EC-87/At.11, 13, and 15) necessary to ensure that conclusions of the safety assessment were valid?

General comments

502. Dr. Nutti and Dr. Squire commented on this question. Given that Dr. Nutti agrees that the request for additional information was necessary, the European Communities will at this stage refrain from commenting on this issue. As regards Dr. Squire, he states that "the requests for further information (EC-87, At 11, 13, 15) appear to be mainly about safety as a feed." This concurs with the lead CA's correspondence specifically mentioning that the request pertained to the safety assessment for animal feed purposes.

Question 42

Given the information before the Panel, including the notification (EC-88/At.1) and additional information provided by the notifier (EC-88/At.10-11), was the information regarding allergenicity, molecular characterisation, and gene transfer in digestive tracts requested by the lead CA (EC-88/At.12) necessary to ensure that conclusions of the safety assessment were valid?

General comments

503. Dr. Nutti does not reply to the question. The question is about the lead CA's very specific request for additional information contained in question contained in EC 88 *Att. 12*. Dr. Nutti instead offers (a less relevant) analysis on the lead CA's request for additional information contained in EC 88 *Att. 9*, to which the applicant provided answers in EC 88 *Att. 11*. Her comments, therefore, must be dismissed. The European Communities will offer a brief reply to the actual question.

Detailed comments

504. Question 42 addressed at the Panel Experts pertained to the questions posed by the lead CA in EC88, Att. 12. The request for additional information contained in that attachment (letter from lead CA of 01. 10.1999) very specifically addresses the risk to humans and animals of potential survival of GM material during digestion in ruminants and subsequent transfer to intestinal cells. Dr. Nutti, however, addresses the questions to which the applicant has already provided answers in EC88, At. 11 and not the questions in EC88, At. 12. Her analysis on the answers in Att. 11, moreover, is non-comprehensive, as she only considers refined sugar as a product for food and animal feed, whereas by-products of sugar production, such as sugar beet pulp, are also used for animal feeding.

505. As regards the actual request for additional information, horizontal gene transfer from GM food and feed in digestive tracts, among others with regard to the use of antibiotic resistance genes (see comments on Q1 and 2), has been debated in forums organized by international organizations since 1990.[191] The same pertains to the digestibility of proteins derived from the expression of transgenes.[192] Recent publications show that a fraction of transgenic proteins and DNA in GM crops, such as the Cry1Ab protein and its gene in GM maize, may survive the intestinal passage after consumption of these GM crops by target animals *in vivo*.[193] Therefore, consideration of the issue

[191] IFBC (1990) Biotechnologies and food: Assuring the safety of foods produced by genetic modification. Regulatory Toxicology and Pharmacology 12: S1-S196;FAO/WHO (1991) Strategies for Assessing the Safety of Foods Produced by Biotechnology, Report of A Joint FAO/WHO Consultation. World Health Organisation, Geneva. http://www.who.int/foodsafety/publications/biotech/1990/en/; FAO/WHO (1996) Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, Rome, Italy, 30 September – 4 October 1996. FAO Food and Nutrition Paper 61. Food and Agriculture Organisation of the United Nations, Rome. http://www.fao.org/es/ESN/food/pdf/biotechnology.pdf ; FAO/WHO (2000) Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, 29 May - 2 June 2000. Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/es/esn/food/gmreport.pdf ; OECD (1993) Safety Evaluation of Foods Derived by Modern Biotechnology. Organisation for Economic Co-operation and Development, Paris. http://www.oecd.org/dataoecd/57/3/1946129.pdf ; OECD (1996) Food Safety Evaluation. Organisation for Economic Co-operation and Development, Paris.; OECD (1998) Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods, Aussois, France, 5-8 March, 1997. Organisation for Economic Co-operation and Development, Paris. http://www.olis.oecd.org/olis/1998doc.nsf/LinkTo/sg-icgb(98)1-final

[192] IFBC (1990) Biotechnologies and food: Assuring the safety of foods produced by genetic modification. Regulatory Toxicology and Pharmacology 12: S1-S196; FAO/WHO (1995) Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology, Report of A WHO Workshop. World Health Organisation, Geneva; FAO/WHO (1996) Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, Rome, Italy, 30 September – 4 October 1996. FAO Food and Nutrition Paper 61. Food and Agriculture Organisation of the United Nations, Rome. http://www.fao.org/es/ESN/food/pdf/biotechnology.pdf.; FAO/WHO (2001) Allergenicity of Genetically Modified Foods. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology. Rome, 22 - 25 January 2001. Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/es/esn/food/allergygm.pdf

[193] Chowdhury, E.H., Shimada, N., Murata, H., Mikami, O., Sultana, P., Miyazaki, S., Yoshioka, M., Yamanaka, N., Hirai, N., Nakajima, Y. (2003) Detection of Cry1Ab protein in gastrointestinal contents but not visceral organs of genetically modified Bt11-fed calves. Veterinary and Human Toxicology 45: 72-75; Chowdhury, E.H., Mikami, O., Murata, H., Sultana, P., Shimada, N., Yoshioka, M., Guruge, K.S., Yamamoto, S., Miyazaki, S., Yamanaka, N., Nakajima, Y. (2004) Fate of maize intrinsic and recombinant genes in calves fed genetically modified maize Bt11. Journal of Food Protection 67: 365-370; Einspanier, R., Lutz, B., Rief, S., Berezina, O., Zverlov, V., Schwarz, W., Mayer, J. (2004) Tracing residual recombinant feed molecules during digestion and rumen bacterial diversity in cattle fed transgene maize. European Food Research and Technology 218: 269-273.

raised by the Belgian lead CA in EC88, At. 12, was highly relevant for the assessment of animal feed safety of GM crops including Roundup Ready sugar beet.

Question 43

Given the information before the Panel, including the notification and additional information provided by the notifier (EC-88/At.10, 11, 13, 14, 15, 16, and 18-26), was the information regarding molecular characterisation and allergenicity of event '77' requested by the lead CA (EC-88/At.27-28) necessary to ensure that conclusions of the safety assessment were valid?

General comments

506. Dr. Nutti comments only on the issue of allergenicity. Her statement that "[the information previously provided by the applicant] on this item covered all the points recommended by WHO/FAO Expert Consultation 1996, and the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003) and its Annex on Possible Allergenicity Assessment" is totally unsubstantiated and misleading as it does not explain whether the request of additional data by the lead CA was scientifically founded. Conveniently, Dr. Nutti omits to mention that also the lead CA's request is entirely in accordance with Codex and FAO/WHO recommendations.

507. Furthermore, the European Communities notes that no expert has provided comment on the request for additional information on molecular characterisation. The European Communities refers to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following).

Detailed comments

508. As she has not provided any explanation, one can only assume that Dr. Nutti's general approach to allergenicity assessments is based on the same assumptions the applicant had made, namely that there are two common characteristics shared by most allergens: first, food allergens are generally abundant in food, usually greater than 1% of the total protein and, second, food allergens are stable to gastric digestion, and a specific test for gastric stability has been established and validated using a broad array of known food allergens.

509. In this view the assessment of allergenicity is possible solely on the basis of *in silico* and *in vitro* parameters, such as sequence homology and stability.

510. However, this view is not adhered to by many other scientists who take the position that more tests, especially using sera from patients and animal models, are needed to assess allergenicity.

511. The answers and requests of the lead CA reflect this debate among scientists in a very scientific way: According to the lead CA the method of homology comparison and sequence comparison as well as stability testing alone is not relevant in all circumstances as, for example, three dimensional epitope characteristics and instability of several known allergens need to be considered. The CA refers to the need to identify possible cross-reactivities using methods such as "RAST".

512. The scientific debate about the assessment of allergenicity has been discussed controversially in international organisations such as OECD for a long time and concluded in increasingly comprehensive models in FAO/WHA expert consultations and CODEX guidelines. The CA reflected

the consequences of these discussions accordingly. The Codex guideline CAC/GL 45-2003, Annex on the Assessment of Possible Allergenicity explains that

> […]sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous epitopes capable of binding themselves specifically with IgE antibodies. A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. Further studies should be conducted as appropriate.[194]

513. Similarly, the 2001 FAO/WHO Expert Consultation on Allergenicity of Foods derived from Biotechnology (Dr. Nutti refers to an older one of 1996) concluded that:

> When the expressed protein comes from a source that is not known to be allergenic, the FAO/WHO 2001 decision tree focuses on (1) sequence homology to known allergens (food and environmental), (2) targeted serum screening for cross-reactivity with sera from patients allergic to materials that are broadly related to the source material for the gene, (3) pepsin resistance and (4) immunogenicity testing in animal models (Annex 4). In this situation the search for homologous allergens is based on two procedures.The first step is a database search for an allergen with a homologous amino acidsequence, according to the principles described in Section 6.1. If this search reveals a level of homology with a known allergen that suggests a potential for cross-reactivity, the expressed protein is considered to be an allergenic risk. No further evaluation for allergenicity would typically be necessary.The second step is conducted if no such homologous protein is found. In such cases,cross-reactivity is tested with a panel of serum samples that contain high levels of IgE antibodieswith a specificity that is broadly related to the gene source (Section 6.3).[195]

514. Finally, the latest FAO/WHO consultation on GM foods (2003) agrees that

> It has been recognized that there is no single parameter that can predict the allergenic potential of a substance. A strategy to assess allergenicity of biotechnology products has been formulated (FAO/WHO, 2001; Codex Alimentarius Commission, 2003), which relies on the parameters: source of the gene, sequence homology, serum testing of patients known to be allergenic to the source organism or to sources distantly related, pepsin resistance, the prevalence of the trait and assessment using animal models. The Consultation recommended that additional efforts should be directed to the further development and validation of models and that there is a need to improve the accessibility and interconnectivity of existing databases or to establish a centralized database on allergenic linear and conformational epitopes and tools for screening transgenes for allergenic potential.[196]

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[194] Section 3.2, Paras. 10 and 11.

[195] 2001 FAO/WHO Expert Consultation on Allergenicity of Foods derived from Biotechnology, http://www.who.int/foodsafety/publications/biotech/ec_jan2001/en/, at para. 5.4.

[196] FAO/WHO Consultation on Food from GM Animals, 2003, http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y5316E/y5316e01.htm, section 5.2.2.3.

515. In the present case, thus, whereas some data on sequence analysis and stability were provided by the applicant, the request of the CA for further allergenicity tests reflected the scientific dispute for a need of improved assessment methods as reflected in turn in the above documents. It is to be noted that the Codex does not provide for a final harmonised and agreed guidance as regards the assessment of allergenicity that would restrict the analysis to the two tests provided for by the applicant.

516. As regards additional information on molecular characterisation, to which no expert has replied, the European Communities would like to offer the following brief comments:

517. In the present case, the lead CA in its request (EC-88/At.27, November 16th 2000) explained very clearly the basis for the request for additional information: lack of quality of Southern blot images and also an expressed concern regarding a fragment observed without sufficient explanation about its nature. On the basis of the available information by the date of the request, the requested information regarding molecular characterisation was, therefore, necessary to ensure that conclusions of the safety assessment were valid.

Question 44

Given the information before the Panel, including the notification (EC-94/At.1-3), was the information requested by the Netherlands(EC-94/At.12) concerning molecular characterization, DNA sequence analysis of the insertion event, analysis of protein levels, effect of glyphosphate treatment, composition, toxicology and the request for a study on dairy cows necessary to ensure that conclusions of the safety assessment were valid?

General comments

518. Dr. Nutti and Dr. Andow both comment on this question in their respective fields of expertise, overlapping on questions 4 and 6, of the lead CA's request. The European Communities disagrees with all of Dr. Nutti's conclusions, as they are either based on erroneous reading of the documents submitted (question 3) or actually inconclusive to the question (questions 4, 5) or unfounded (question 6). The European Communities further disagrees with one aspect of Dr. Andow's analysis of question 6 of the lead CA's request.

519. Furthermore, as no one has offered comments on questions 1 and 2 of the lead CA's request, the European Communities will offer a brief explanation.

Detailed comments

520. Dr. Nutti's comments on question 3 of the CA's request are based on an error as she takes the applicant's reply to that request to be the data originally submitted.

521. Dr. Nutti concludes that the information was not necessary to ensure the validity of the safety assessment, as "the explanation given by the notifier was acceptable, since in EC-94/ *At 13*, the

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Notte that even the present strategy based on a sequence prediction model established by FAO/WHO was shown to be amenable to refinement by important groups (for example, Stadler and Stadler, Allergenicity prediction by protein sequence, FASEB, J 2003, 17, 9, 1141-3; Kleter and Peijnenburg, Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE - binding linear epitopes of allergens. BMC Struct Biol, 2002, 2, 1, 8; and Zorzet et al., Prediction of food protein allergenicity: a bioinformatic learning systems approach, In Silico Biol, 2002, 2, 4, 525-534). Difficulties in the concept are summarised in Jank and Haslberger, Improved evaluation of potential allergens in GM food, Trends Biotechnol., 2003, 21, 6, 249-250).

notifier presents the results of second year study in order to test this assumption, and the results confirmed that the expression levels were similar."

522. Indeed, the data presented by the applicant in At. 13 are precisely the missing data that the CA had asked for. These data were submitted in 2002. The CA's request dates from 2000.

523. Implicitly, therefore, Dr. Nutti confirms that the request of the lead CA was justified and that the additional information, thus, was necessary to reach a valid conclusion on this issue.

524. As regards question 4 of the lead CA, Dr. Nutti and Dr. Andow address different aspects of that question in their respective comments.

525. Dr. Nutti comments relate to the necessity of comparing herbicide sprayed and non herbicide-sprayed GM maize. The lead CA had asked for such data in order to verify whether the herbicide-sprays could have influenced the results of compositional data. The applicant, in its original application had provided some data that collectively compared both sprayed and non-sprayed samples with other groups of samples (compositional data EC94; At.2, pp. 76-186; request EC94, At.12).

526. Dr. Nutti in her comment mentions that the comparison of GM with non GM crops usually does not entail herbicide treatment of the GM crop. She also refers to the *post-hoc* information provided by the applicant (EC94, At.13) indicating that in other GM crops (GA 21 maize, Roundup Ready soybean), no effect of herbicide treatment on compositional data had been noted. In addition, she concludes that the data on both herbicide-sprayed and non-sprayed had been provided and that therefore the requested additional information was not necessary for the safety assessment.

527. However, the arguments provided by Dr. Nutti are actually in support of the lead CA's request for a verification of the absence of an effect of glyphosate. Since data on both sprayed and non-sprayed samples are already available, spraying constitutes a variable and the data can be compared to check whether this variable causes differences or not, as has been previously been done for other GM crops.

528. This issue is also addressed by the Codex Guideline 2003 under the heading Compositional Analysis of Key Components, in which the comparison of a GM crop with both its sprayed and non-sprayed counterparts is recommended:

> Analyses of concentrations of key components of the recombinant-DNA plant and, especially those typical of food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (*e.g.* application of an herbicide).[197]

529. Treatment of a GM crop (MaisGard Roudup Ready using HT and Bt genes stacked) with applied herbicide (glyphosate) could trigger metabolic changes in the GM maize event. The effects on plant metabolism under regional conditions of the herbicide applied to this GM crop event were unknown at the time. Thus toxic effects on non target organisms likely to be exposed to the herbicide treated GM maize event (*e.g.* humans, dairy cows and a range of other herbivores consuming glyphosate-treated Maisgard Roundup Ready) were impossible to predict without testing. Therefore, comparative data on treated and untreated GM maize was required to assess such effects.

[197] Codex guideline CAC/GL 45-2003, para. 44

530. Dr. Andow's comment on question 4 relates to the method of the statistical analysis requested by the lead CA. As Dr. Andow rightly points out, with the statistical design and analysis used by the notifier, it would not be possible to estimate an RR effect independent of a glyphosate effect (see Dr. Andow's comments at para. 44.07). The data provided thus, is inadequate to reveal interactions between the GM crop, local environment, herbicide treatment(s) and any non-target organisms likely to be exposed to GM gene products, the applied herbicides and their metabolites induced in the HT crop after glyphosate treatment.

531. In conclusion the request by the lead CA for an additional statistical analysis to verify whether herbicide sprays may have caused additional changes in composition was therefore justifiable and necessary to reach a conclusion on the safety of maize MON810xGA21.

532. In her comments on question 5 in the lead CA's request, Dr. Nutti addresses two different issues, namely the request for additional comparisons of hybrid MON810xGA21 with non GM lines and the request for data on additional secondary metabolites and antinutrients.

533. As regards the request for additional comparisons of hybrid MON810xGA21 with non GM lines, Dr. Nutti refers to "different approaches" that can be used to perform comparisons based on the compositional analysis on a hybrid GM. She takes the view that the one opted for by the applicant, i.e. to compare the hybrid only to its parental lines (but not to conventional counterparts, providing such a comparison has already been carried out only between the parental lines and conventional counterparts) is sufficient.

534. The lead CA, on the other hand, has taken the view that a (direct) comparison of the hybrid with conventional counterparts would also be required.

535. In fact, the issue of exact data requirements for conventional crosses of two parent GM lines is still being discussed in and outside the EU. Comparison of the different national legislations pertaining to GM crops shows that there are differences with regard to the scientific understanding of the needs for further risk assessments, and with regard to the obligation to notify conventional crosses of GM lines or not.[198] The issue is not specifically addressed by the Codex guideline.

536. The safety of the parent lines is important, because the same genetic modifications can be present in the hybrid as in these GM parent lines. However, information on the parent lines alone cannot provide full reassurance that the cross will be safe as well. For example, the introduced genetic material and the products that are derived from this genetic material (*e.g.* enzymes), may interact with each other within the conventional cross of GM lines.

537. For example, new genes may participate in different steps in the same pathway (cascade) of enzymatic reactions (such as, for example, the formation of a vitamin or plant fibre). In case these genes are introduced into separate GM crops, which are subsequently crossed with each other, the cross may exhibit features that are distinctive from the parental lines (for example, this has been observed by Pincon et al. (2001) in GM tree plants with modified production of precursors for cell fibre components).[199] Another example is that of "gene silencing" (inactivation of gene function) by

[198] Kuiper, H.A., Kleter, G.A., Noteborn, H.P.J.M., Kok, E.J. (2001) Assessment of the food safety issues related to genetically modified foods. Plant Journal 27: 503-528.
http://www.blackwellpublishing.com/plantgm/tpj1119.pdf

[199] Pincon, G., et al. (2001) Simultaneous down-regulation of caffeic/5-hydroxy ferulic acid-O-methyltransferase I and cinnamoyl-coenzyme a reductase in the progeny from a cross between tobacco lines homozygous for each transgene. Consequences for plant development and lignin synthesis. Plant Physiology 126: 145-155.

bringing together genes with similar sequences from different parents within the same plant (as observed, for example, by Charrier et al. (2000) and De Wilde et al. (2001)).[200] Interestingly, the British CA asked for data on the homology of the transgenic constructs inserted into the parental GM maize lines MON810 and GA21 (EC94, At. 10). This information would also have been particularly relevant for the latter example of "gene silencing", for which there would be a likelihood of occurrence in the crossed line MON810xGA21 in case of homology between the constructs.

538. In exhibit 94/013, answer to question 1, clause B, second last bullet point, the notifier states:

> Finally, there are no known mechanisms by which two inserts at different locations on different chromosomes could stimulate recombination in each other (if they do not express proteins involved in recombination pathways).

539. While this statement is basically correct, it is also misleading because it gives the impression that only recombination in the functional inserts is relevant for the risk assessment. However, in plants the genes interact with other genes in sometimes very complex patterns involving genes distantly located on the same or other chromosomes. It is therefore possible that recombination may occur among or within genes at other loci, and that these genes may interact with an inserted gene. Furthermore, the size of sequence motifs involved in recombination events may be short. It can not be excluded that small random unintended and undetected insertion events can be present in the parental GM maize events MON 810 and GA21. To ensure that the hybrid GM maize is safe, it is therefore necessary to test the hybrid separately, and to compare it not only with the GM parental events but also with conventional counterparts.[201]

540. While this issue is not specifically addressed in the Codex Guideline 2003 some support for the lead CA's approach can be found there as well. Thus, whereas in para.44 of the Guidelines it is recommended that:

> "The comparator(s) used in this assessment should ideally be the near isogenic *parental* line"[emphasis added][202]

541. It is also recommended that:

> Analyses of concentrations of key components of the recombinant-DNA plant and, especially those typical of food, should be compared with an equivalent analysis of a *conventional* counterpart grown and harvested under the same conditions."[203] [emphasis added]

542. The Codex guideline defines a conventional counterpart as:

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[200] Charrier, B., Scollan, C., Ross,S., Zubko, E., Meyer, P. (2000) Co-silencing of homologous transgenes in tobacco. Molecular Breeding 6: 407-419; De Wilde, C., Podevin, N., Windels, P., Depicker, A. (2001) Silencing of antibody genes in plants with single-copy transgene inserts as a result of gene dosage effects. Molecular Genetics and Genomics 265: 647-653.

[201] See Wilson et al 2004; Technical report for EcoNexus; Freese and Schubert 2004 Biotechnology and Genetic Engineering 21: 299-324; The Committee on Identifying and Assessing Unintended Effects of Genetically Engineered foods on human health (2004). "Safety of Genetically Engineered Foods: Approaches to assessing Unintended Health Effects". Publishers: The National academic press, Washington, D.C. National academy of Sciences (purchasable pdf download from www.national-academies.org).

[202] Codex guideline CAC/GL 45-2003 para. 44

[203] Ibid.

> a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food.[204]

543. In the present case, the GM parental lines cannot be considered "conventional counterparts" with a history of safe use according to these standards, since at the moment of the request by the Dutch lead CA, MON810 had only been approved for several years in the EU, and GA21 was still in the approval procedure. Therefore, the comparison with the GM parental lines was not sufficient and the request for a comparison of the hybrid MON810xGA21 with non-GM lines was justifiable.

544. More generally, data regarding the safety of the biotech parent plants are obviously relevant to the safety assessment of the hybrid plant products. However, while relevant, they are not sufficient.

545. Note also that this approach has recently been confirmed from a molecular characterization point of view by an US EPA-FIFRA SAP panel report[205] which addressed this general problem in the context of a specific new product (Bt Cry1F/Cry1Ac). The US expert panel stated that:

> A molecular characterization of the new product should show that the recombinant traits/ sequences in the new product are identical to the insertion /traits in their parental lines. In principle, the method of breeding the two parental lines could affect the genetic characteristics of the inserted traits. Therefore, evidence is needed that no such effect has occurred. For these products, a risk assessment combining *evidence from parental lines and the product should be requested*.

546. As already explained in the previous comments, it can not be excluded that unintended effects may result from hybridisation between the two parental GM events. The data requirements for assessment of individual transformation events do not normally include provisions for experimental data on the effect of hybridisation between the event under study and other GM events previously authorised or under separate evaluation. This may be justifiable given the assumption that such unintended hybrids will only occur in low frequency under field conditions, and consequently the exposure from these hybrids to humans, animals and the environment will be low. On the contrary, authorised hybrids will by default occur at high frequency and consequently the exposure will be significantly higher. Consequently, the intended hybrid must be subject to separate evaluation including additional data from appropriate feeding trials.

Dr. Nutti also mentions that the comparison with non-GM parental lines may sometimes be problematic because they might not have been developed. In fact, the original study on the compositional data (EC94, At.2, pp. 76-186) mentions that besides the hybrid MON810xGA21, the parental GM lines MON810 and GA21, also non-GM control lines and commercial reference lines were grown in the same locations (EC94, At. 2, p. 82). However, compositional data of these additional non-GM lines have not been included in the statistical comparison by the applicant. Therefore, Dr. Nutti's concern about the possible unavailability of non-GM material for a comparison of MON810xGA21 with non-GM maize lines does not apply to this case.

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[204] Codex guideline CAC/GL 45-2003 para. 8

[205] SAP Report No. 2004-05. MINUTES of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) Meeting, June 8-10, 2004, Arlington, Virginia. A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management For Bacillus thuringiensis (Bt) Cotton Products. 84 pages.

547. As regards the lead CA's request for data on additional secondary metabolites and antinutrients Dr. Nutti's assessment is being contradicted by the standard set out in Codex Guideline CAC/GL 45-2003.

548. The lead CA requested data on five additional, secondary substances that could provide an indication of potential unintended alterations of metabolic pathways. This would supplement the data that had already been provided for the secondary substance phytic acid, which also acts as an "antinutrient" since it interferes, for example with mineral uptake from the intestines.

549. Dr. Nutti concludes that this additional analysis would not have been necessary, since the previous compositional data provided by the applicant indicated that the pathways leading to these substances had not been affected. For example, levels of phytic acid, which is formed from inositol (one of the recommended substances), had not been altered in MON810xGA21 maize.

550. In the Codex Guideline CAC/GL 45-2003, this issue is dealt with under "Unintended effects" and under "Potential accumulation of substances significant to human health".

551. Thus paragraph 15 of the Codex Guideline CAC/GL 45-2003 states that

> Unintended effects may also result in the formation of new or changed patterns of metabolites.

552. Paragraph 54 of the Codex Guideline states:

> Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g., procedures for assessing the human safety of chemicals) should be applied.

553. Based upon these standards, the request of the lead CA was relevant from the point of view of safety of the GM crop and any plant secondary metabolites that have been changed as an unintended side-effect of the genetic modification.[206]

554. It may be noted in passing that the applicant has also provided data on such plant secondary metabolites in a study on another GM maize line.[207]

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[206] Specifically from an environmental point of view unusual crop phenotypes arising from unintended genetic changes could have a significant impact on one or more ecological interactions of the particular GM cultivar (event). This is because crop plants produce a multiplicity of bio-active compounds that are individually or as a mixture associated with positive and negative nutritional qualities when consumed by non target organisms associated with the crop in any particular region and local agro-ecosystem. Because of unknown or unpredictable dose responses to bio-active compounds in plants and in general, "hormesis demands a reappraisal of the way risks are assessed" (Calabrese and Baldwin, 2003; Toxicology rethinks its central belief. Nature 421, 691-692). The Codex Guidelines for GM foods includes analysis of unintended and indirect effects on the environment due to increasing evidence of unanticipated changes in biologically-active plant compounds arising unexpectedly in transgenic crops. (e.g. Shewmaker et al., 1999; Saxena & Stotsky 2001; Birch et al., 2001; Haslberger, 2003

[207] Monsanto (2002) Safety Assessment of Roundup Ready Corn Event NK603. Monsanto Co, St. Louis. www.monsanto.com/monsanto/content/sci_tech/prod_safety/roundup_corn/pss_NK603.pdf; Ridley,

555. Finally, as regards question 6 of the lead CA's request, the European Communities disagrees both with Dr. Nutti's and Dr. Andow's comment.

556. In question 6, the lead CA considered that the method to genetically modify the parental maize may have caused the unintended insertion of additional fragments into the maize DNA. Shortly before the lead CA's request, it had become known that Roundup Ready soybeans (developed by the same applicant, Monsanto), which had previously been approved in the EU for food and feed purposes, but contained additional DNA fragments. These previously undetected fragments had been revealed by molecular techniques to sequence DNA inserts.

557. Dr. Nutti concludes that the information that had already been submitted by the applicant was sufficient for the safety assessment. With regard to the toxicology studies, she refers to studies (*e.g.* acute toxicity, *in vitro* stomach fluid digestion) that have been performed with purified proteins and not with the whole maize product. However, whole feed/food studies should be performed in order to assess the potential health consequences of unintended effects, such as those may have been caused by the insertion of additional DNA fragments, beyond the intended modifications (e.g. transgenic proteins) that have been tested for toxicity. (See also EC Comments on Question 44bis).

558. Dr. Andow acknowledges the lead CA's concern about additional DNA fragments are "valid concern," (Dr. Andow's reply, para. 44.10), but comments in this regard that "it would seem that the notifier would have other alternatives to the toxicity test to address the concern of the Netherlands CA." (*ibid.*). However, Dr. Andow does not give the details of the alternative techniques that he claims may address the concerns of The Netherlands. These methods should therefore provide reassurance about additional fragments having been inserted or not.

559. In literature, other ways to detect unintended effects are described, such as the use of advanced analytical technologies called "profiling" or "metabolomics". These techniques are still under development and have not been validated yet for routine application in the safety assessment of GM foods.[208]

560. As previously mentioned, there has already been a post-market discovery of an unintended effect, *i.e.* additional DNA fragments having been inserted into GM herbicide-resistant (Roundup Ready) soybean. In this case, the applicant (Monsanto) itself argued that these additional fragments could not pose a risk, since no adverse effects had been observed in previously published animal feeding studies with whole feed products derived from Roundup Ready soybeans.[209]

561. The lead CA made its request shortly after the new information surrounding the issue of fragments in GM soybean had become public. For this reason, as well as those given above, it is justifiable that the lead CA asked an additional animal feeding experiment with the whole product as additional reassurance for the absence of adverse effects that might have been caused by unintended insertion of additional DNA fragments.

W.P., Sidhu, R.S., Pyla, P.D., Nemeth, M.A., Breeze, M.L., Astwood, J.D. (2002) Comparison of the nutritional profile of glyphosate-tolerant corn event NK603 with that of conventional corn (Zea mays L.). Journal of Agricultural and Food Chemistry 50: 7235-7243.

[208] Chassy, B., Hlywka, J.J.,. Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Re, E.B., Reid, J.E. (2004) Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology, Prepared by a Task Force of the ILSI International Food Biotechnology Committee. Comprehensive Reviews in Food Science and Food Safety 3: 35-104. http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

[209] Monsanto (2000) Updated Molecular Characterization and Safety Assessment of Roundup Ready Soybean Event 40-3-2. Monsanto Co. St. Louis, 20 http://archive.food.gov.uk/pdf_files/acnfp/summary.pdf

562. Finally, the European Communities would like to briefly comment on the lead CA's request for additional information in questions 1 and 2 of At. 12, on which none of the experts has commented.

563. The European Communities would refer to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following). In the present case, it should be noted that the notifier applied only Southern blots to establish the number and composition of insert(s). However, by the date of the notification (EC-94/At.1-3, February 29th 2000) DNA sequencing was a standard method that could be applied to verify the composition of the insert. To request such information was therefore necessary and justified.

Question 44bis

Given the information before the Panel, including the first whole food study and agronomic performance tests (EC 94/At.3, pp. 40), was a second animal whole food study (EC 94/At.12) necessary or useful to identify potential adverse effects, including those associated with small random DNA insertions, that had not been previously identified?

General comments

564. The question refers to question 6 of the lead CA in its request of July 2000 (At. 12), which was already covered by and addressed in question 44. Dr. Nutti's comments are identical to her comments in question 44. Therefore, the European Communities refers to its above comments under question 44.

Question 44ter

In the context of this corn product which was produced through conventional breeding from biotech parents, is data regarding the safety of the biotech parent plants relevant to the safety assessment of the hybrid plant products? Given the results of the risk assessment studies of the biotech parents, were additional studies necessary or useful to ensure that the conclusions of the safety assessment related to the hybrid plants were valid?

General comments

565. The question can be linked to question 5 of the lead CA in its request of July 2000 (At. 12), which was already covered by and addressed in question 44. Dr. Nutti refers back to her comments on that issue in question 44 and so does the European Communities.

Question 45

Given the information before the Panel, were there scientific or technical reasons which explain why the process of validating the detection method took at least fourteen months (June 2002-August 2003)? (See EC-91/At.49-56)?

General comments

566. Dr. Nutti acknowledges that detection methodology is not her main area of expertise. Question 45 turns in fact on an overall delay of approximately 14 months. No specific expertise on detection methods is actually required to see that the applicant took more than 9 months out of these 14 months to submit any information (methods or materials) whatsoever (EC-91, At. 50). That that

information could not even be used to start work on the EU side is something Dr. Nutti does not seem to have identified. Her "*impression*, that there were no scientific or technical reasons which explain why the process of validating the detection method took at least fourteen months" is simply a wrong impression as will be explained in more detail below.

Detailed comments

567. The process of method validation for GMO detection methods is both complex and time consuming. Simply put, two sets of information are needed to develop a detection method. First, the actual method (i.e. the protocol) is needed. The method consists of two components: (a) a method to extract good quality of DNA and (b) a method to assess the amount of GM analytically. Second, control samples are needed. Control samples serve as an internal control for any analytical method. Provision of precise *control materials/samples* for precision instrument calibration must be *simultaneously* supplied by the notifier along with the detailed method description. Thus, the development of the detection method cannot be executed without appropriate control samples. For GMOs they can only be supplied by the applicant who constructed the transformed organism.

568. With respect to the present product in question (Monsanto Roundup Ready corn GA21) following initial contact with the notifier on 5 June 2002 (EC-91, At. 45) to discuss and agree the method validation for GA21, the applicant and the Joint Research Centre signed a Material Transfer Agreement on February 20th 2003 (EC-91, At. 49) and the applicant did not provide *any* of the above information before 27 March 2003, i.e. approximately 9 and a half months after the initial contact date.

569. Furthermore, and contrary to what Dr. Nutti claims, the information submitted on that date, was incomplete with regard to both of the above sets of information needed. Therefore, the validation process could not to be started.

570. On the one hand, the information submitted was incomplete with regard to the method description and this in several respects. First, the applicant failed to provide component (a) as described above, i.e. a method on how to extract the DNA from the control samples, which is in the first step in DNA based GMO detection and quantification. Without that information no method validation can start. The missing information with respect to a suitable method for the extraction of DNA of good quality was actually provided by JRC: between April and July 2003 (EC-91, At. 56), the JRC contributed to the further scientific and technical definition and completion of this method through the supply of a draft technical protocol for DNA extraction for subsequent DNA quantification and method application, thus completing the system in readiness for validation to begin proper.[210]

571. Second, certain critical reagents (i.e. chemical substances needed as ingredients in the analysis) provided by the notifier for the purposes of the validation procedure were found to be supplied not only in insufficient quantity, but faulty (EC-91, At. 59), further delaying progress.

572. Finally, the company failed to provide convincing empirical data regarding overall method performance, a prerequisite of method acceptance (EC-91, At. 52).

[210] It should be noted that the European Commission – Joint Research Centre bore all the costs associated with this validation procedure.

573. On the other hand, the information submitted on 27 March 2003 was incomplete with regard to the control samples. The applicant provided the appropriate amount and quality of control samples only 9 April 2003 (EC-91, At. 51)

574. It was not until July 2003, the information necessary to launch the validation process was complete.

575. As soon as this was the case, the JRC launched an in-house validation in its own facilities, followed by a pre-validation trial with three laboratories which took place between December 2003 and January 2004, and the collaborative trial took place between April and June 2004.[211] Dr. Nutti does not comment on these delays, which are extraordinarily short delays given the complexity and dimension of this process.

Question 46

Were detection methods commercially available in 2002 sufficient to enable the detection of the transgenic proteins expressed by the plant line Bt11 sweet corn?

General comments

576. Both Dr. Nutti and Dr. Healy comment on this question. The European Communities has already explained the need for an *event specific* as opposed to a *trait specific* detection method for GMOs. Nobody would contest that there was *no commercially available event specific detection method for Bt11 sweet corn in 2002*. Such a method was only recently published in a peer reviewed journal in April 2003,[212] and modified for event specific quantitative application (published on 22 May 2004),[213] while the validated study of this method was made available on the internet pages of the Community Reference Laboratory on 28 July 2004.[214]

577. It should be stressed that what both experts discuss here, are generic *trait specific methods*, i.e. methods that allow only for the detection of a specific expressed trait (such as the CryIA(b) protein). Trait specific methods cannot distinguish one GMO product (i.e. transformation event) from another if both are genetically modified with the same trait (such as for example Bt 11 sweet corn and Bt176 maize. Therefore, whether or not trait specific methods existed for CryIA(b) and the PAT gene in 2002 is irrelevant , as they would not have served to specifically detect and identify Bt11 sweet corn as such.[215]

Question 47

Given the information before the Panel, were there scientific or technical reasons which explain why the process of validating the detection method took twelve months (EC-92/At.54-56, 57-65, and 66)?

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[211] Information has been made publicly available on 17 January 2005 on the Internet pages of the Community Reference Laboratory (http://gmo-crl.jrc.it)

[212] Rønning, S.B., Vaïtilingom, M, Berdal, K.G. & Holst-Jensen, A. 2003. Event specific real time quantitative PCR for genetically modified Bt11 maize (Zea mays). Eur Food Res Technol 216: 347 – 354

[213] Hernández M., Duplan M-N, Berthier G., Vaitilingom M., Hauser W., Freyer R., Pla M. and Bertheau, Y. Development and comparison of four RTi-PCR systems for specific detection and quantification of Zea mays L. J. Agric. Food Chem. 2004. 52: 4632-4637

[214] Information has been made publicly available on 28 July 2004 on the Internet pages of the Community Reference Laboratory (http://gmo-crl.jrc.it).

[215] In any event it may be pointed out that while it is correct to say that there was a (trait specific) detection method for CryIA(b) in 2002 this method had not yet been validated. As for a detection method for the PAT gene, as discussed in Question 17, no such method exists to-date.

What would constitute an adequate amount of material to be used in the detection method validation? What are adequate performance indicators for pre-validation results for this product?

General comments

578. As for question 45, Dr. Nutti here acknowledges that detection methods are not her main field of expertise. Indeed, this time she openly admits that she has "no expertise or references to answer about adequate amount of material to be used in the detection method validation and adequate performance indicators for pre-validation results for this product." In light of this, her conclusion that there were no scientific or technical reasons which explain why the process of validating the detection method took twelve months is clearly unsubstantiated. For, in order to be able to come to such a conclusion, one precisely needs to have some knowledge about "adequate amounts of material" and "adequate performance indicators." Dr. Nutti herself establishes that all exchanges between the applicant and the JRC (At. 56 through 66) were about the adequacy of the data submitted. As she does not have the relevant expertise, her reply is, therefore, unfounded and must be dismissed.

Detailed comments

579. The European Communities refers to its comments in question 45: For a detection method to be developed, two sets of information are needed. (1) The protocol (method) including (a) a DNA extraction method and (b) an event specific quantification method, and (2) control samples.

580. At its first meeting with the European Commission on 24 June 2002 the applicant confirmed that they had a method ready and agreed to supply the necessary materials (exhibit 92/54). However, materials (in this instance DNA) necessary to start the validation procedure was only received on 22 January 2003, some 7 months after first contact with the European Commission, and 3 months following a planning meeting (held on 23 October 2002), in which details of the validation and materials (DNA from seed) which should be provided by were agreed.

581. However, by 28 January 2003 quality testing of this DNA material employing a range of testing instrumentation by the JRC revealed either very low amounts or, in extreme cases, virtually no DNA material (i.e. a virtual absence of DNA) in the tubes provided. This is very clear from exhibit 92/56. Such measurements were considered atypical, especially from maize seed material, and indicated a technical failure (and hence low confidence with the material). Following some additional guidance from the JRC on 30 January 2003, the applicant provided a replacement batch of DNA material on 27 February 2003 (exhibit 92/58), exhibiting adequate quantities in the expected concentration range.

582. All materials and information was then complete to commence the pre-validation study, which was subsequently launched on 10 March 2003, some 8 and a half months following the initial meeting between the European Commission and the applicant. The pre-validation study was completed 51 days later on the 30 April 2003, the results of which indicated the proposed method to be unsuitable to proceed (exhibit 92/59). In fact, the results indicated an inherent problem with the detection system, experienced by all participating laboratories in the ring-trail and further indicated that the applicant had not taken the preparative steps as agreed at the planning meeting in October 2002 to optimise the method.

583. On the 23 May 2003, following discussions with the QPCRFOOD[216] consortium, the JRC proposed the use of a successfully pre-validated Bt11 event-specific quantitative method as

[216] QPCRGMOFOOD EU share cost action project (contract no. QLK1–1999–01301).

developed, and offered, by the consortium. Based on the pre-validation results, it was clear that this method did not exhibit the same inherent technical limitations as the original applicants system (exhibit 92/60). After reviewing the details, the applicant agrees on 7 July 2003 to adopt the QPCRFOOD method (exhibit 92/63) and, following receipt of requested reagents on 15 July 2003 (exhibit 92/65) the JRC launches a full validation study on 30 July 2003 (almost 13 months following initial contact with the applicant, but just 3 weeks after the decision to proceed with the better QPCRFOOD method was agreed by the applicant). The JRC reported the positive result of the validation study on 2 October 2003 (exhibit 92/66), in a little over two months from proper commencement.

Question 49

Given the information before the Panel, including the application (EC-93/At.1-2), was additional information regarding nutritional and biochemical characterization and toxicity of the transgenic plant requested by the Greek and Italian authorities (EC-93/At.16-17) necessary to ensure that conclusions of the safety assessment were valid?

General comments

584. Dr. Nutti concludes on almost all specific requests that they were necessary. It is only with regard to questions related to the herbicide treatment that she finds the CA's requests unnecessary based on the "understanding that the risk assessment conducted here is related to GMO and not to the herbicide." The potential effects of herbicide treatment on crop characteristics, however, are highly relevant to the safety assessment of herbicide-tolerant GMOs. Dr. Nutti, therefore, is wrong in assuming that such information would not be necessary, just because the risk assessment is performed on the GMO and not on the herbicide itself. Finally, in commenting on the necessity of carrying out studies of sub-acute toxicity, Dr. Nutti -confirms the CA's initiative to ask for more information.

Detailed comments

585. Dr. Nutti correctly asserts in her review of Italian CA's questions 1 and 2 that herbicide *residues* have to comply with standards like those of the Codex alimentarius. However, information about herbicide *treatment* of the GM crop was also requested in question 1. This is equally relevant for the safety assessment of GMOs, since it provides information about the *conditions in which the tested crops have been cultivated.* In fact, agronomic practices, including herbicide treatment, of GM crops used for compositional analysis are also addressed by Codex guideline CAC/GL 45-2003 which states:

> Analyses of concentrations of key components of the recombinant-DNA plant and, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (*e.g.* application of an herbicide).[217]

586. Information on herbicide treatment therefore constitutes a relevant part of the data on the field trials from which samples for the compositional analysis have been obtained.

587. Question 2 of the Italian CA pertained to the "nature of possible residues" derived from herbicide treatment. Whereas Dr. Nutti correctly notes that the levels of these residues should be

[217] Codex guideline CAC/GL 45-2003, para. 44

within the limits specified with standards, the question is relevant from a safety point of view, regardless whether the issue falls under a different legislation or not.

588. In fact, the Codex guideline CAC/GL 45-2003 also considers this issue under the heading "Potential accumulation of substances significant to human health":

> Some recombinant-DNA plants may exhibit traits (e.g. herbicide tolerance) which may indirectly result in the potential for accumulation of pesticide residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances which may be relevant to human health. The safety assessment should take this potential for accumulation into account. Conventional procedures for establishing the safety of such compounds (e.g., procedures for assessing the human safety of chemicals) should be applied.[218]

589. The questions are therefore relevant from the point of view of safety of the GM crop and any pesticides that might accumulate in it.

590. On the issue of sub-acute toxicity studies, the Italian CA requested such information in order to obtain a more detailed assessment of the toxicological aspects.

591. The animal experiments that had been summarized by the applicant in its application (EC-93, At. 2) include a chicken broiler feeding study (nutritional) and a repeated-dose acute toxicity study (14 days) with the purified transgenic protein administered to in rats. Dr. Nutti concludes that if the results of the composition analysis of Liberty Link soybean, as well as the acute toxicity animal study that have been carried out with it, were satisfactory, no additional sub-acute animal test would be needed. However, in the absence of full data on these tests, she says to be unable to judge the request.

592. In fact, this inability to judge these data due to their incompleteness at the time of the Italian lead CA's request justifies the request for an animal study. This issue is also addressed by the Codex guideline CAC/GL 45-2003:

> If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, properly designed animal studies could be requested on the whole foods.[219]

593. At the time of the request, it was therefore relevant to ask for an additional animal toxicity study.

Question 51

Given the information before the Panel, including the application (EC-95/At.1-2) and the responses from Pioneer/Dow AgroSciences (EC-95/At.10-12), was information regarding molecular characterization, compositional analyses and toxicological analyses of the product requested by the Health Council of the Netherlands (EC-95/At.8 and 13) necessary to ensure that conclusions of the safety assessment were valid?

[218] Codex guideline CAC/GL 45-2003, para. 54
[219] Codex guideline CAC/GL 45-2003, para. 11.

General comments

594. Dr. Nutti addresses issues of compositional analysis and toxicological analysis. While she confirms the necessity of requesting data on the levels of certain nutritional elements and secondary metabolites, she does not see the need to ask for three (instead of two) seasons of field data, a view which she does not substantiate and which is not supported by Codex standards. Her comment on the broiler chicken study seems misplaced as there was actually no request for such data in the attachments under consideration. Furthermore, the European Communities disagrees with Dr. Nutti's conclusion on the necessity of a 90-day toxicity study.

595. Finally, as Dr. Nutti has not addressed the issues relating to molecular characterization, the European Communities will offer some brief comments.

Detailed comments

596. First, on the number of seasons for compositional data (question 2 of the lead CA), Dr. Nutti notes that, whereas two seasons are generally acceptable, the Dutch CA asked for compositional data from three seasons. Actually, the Codex guideline CAC/GL 45-2003 addresses this issue by stating that field trials for compositional analysis should be carried out during a sufficient number of generations, but does not mention a specific number of generations (or seasons):

> Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature.[220]

597. At the time of the request, the applicant had provided data from the seasons 1998-1999 in Chile (Southern hemisphere) and 1999 in France and Italy (Northern hemisphere). The lead CA considered this to be one season (EC95, At.8). Given this opinion of the CA and the Codex guideline CAC/GL 45-2003 recommendation on this issue, it therefore appears justifiable that, at the time of the request, the Dutch CA asked for data from multiple (3) seasons.

598. In addition, the Dutch CA later (in reply to additional compositional data provided by the applicant) asked for an additional study from the US, as this would be representative for the commercial production of the new maize line.[221]

599. Furthermore, the lead CA's request is in line with Codex guideline CAC/GL 45-2003, paragraph 45, pertaining to compositional analysis of key components, which reads as follows:

> The location of field trials should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown.

600. Therefore, the data requested by the lead CA on compositional analyses from multiple (3) seasons, as well as from regions representative of commercial maize cultivation (and export to the EU) are in line with Codex recommendations and relevant to the safety assessment.

601. Second, Dr. Nutti comments on the necessity of additional data on a broiler chicken study. There was no question on the chicken broiler study posed in EC95, At. 8 and At. 13, neither was the broiler study mentioned in the attachment, namely EC95, At. 11. Dr. Nutti's comment on the chicken broiler study, therefore, cannot be verified, since no reference to this study could be found in the

[220] Codex guideline CAC/GL 45-2003, para. 45.

[221] Note that this issue was also dealt with under question 35, where Dr. Andow cited trade statistics showing that the major nations exporting maize were the US, Argentina, Brazil, and the People's Republic of China (see Dr. Andow's reply, para. 35.03).

documents mentioned [requests by Member State CA (EC95, At. 8 and 13) and reply from the applicant (EC95, At. 11)].

602. Third, Dr. Nutti seems to argue that the 90 day rat study requested by the lead CA was not necessary as "additional testing will be required if the composition is not comparable to conventional foods, which is not the case of Pioneer/Dow AgroSciences Bt corn Cry1F (1507)."

603. At the moment of the request of the Dutch CA for an additional 90-day rat study, the applicant had provided (summaries of) a nutritional study in chicken broilers fed whole products and acute toxicity in rats with the purified transgenic proteins Cry1F and PAT. It should be stressed here that the chicken broiler study is not a model for toxicology, but for nutrition (for example, see the discussion on this issue in Chassy et al., 2004). During studies on broilers, the growth, bodyweight, feed consumption, and weight and composition of edible parts after slaughter are usually measured. Therefore, the toxicity animal studies had been limited to those on acute toxicity of purified Cry1F and PAT, and no toxicity studies with the whole food.

604. According to Dr. Nutti, the additionally requested 90-day toxicity study was not necessary, since compositional equivalence had been demonstrated. The rationale provided by the Dutch CA for its request for the 90-day study was that it would provide additional reassurance of no unintended changes with adverse effects (EC-95, At.8).

605. Whole feed/food studies can be used in order to provide reassurance about the potential health consequences of unintended effects, such as those that may have been caused by the insertion of additional DNA fragments, beyond the intended modifications (e.g. transgenic proteins) that have been tested for toxicity. In fact, other ways to detect unintended effects, such as the use of advanced analytical technologies called "profiling" or "metabolomics", are in development, but have not been validated yet for routine application in the safety assessment of GM foods.[222]

606. An example of animal feeding studies serving as additional reassurance of no adverse effects is Roundup Ready soybeans. It was discovered in 2000 that unintended insertions of DNA fragments had occurred in these soybeans, which had already been approved as a GM food in a number of countries, including the EU. The applicant (Monsanto) itself argued that these additional fragments could not pose a risk, since, among others, no adverse effects had been observed in a previously published peer-reviewed animal feeding study with whole feed products derived from Roundup Ready soybeans.[223]

607. It is therefore justifiable of the lead CA that, in the absence of toxicity studies with whole foods, it sought additional reassurance for the innoxiousness of any unintended effects by asking for an additional animal toxicity experiment.

608. Finally, as regards the CA's requests for additional molecular characterization (on which Dr. Nutti has not commented), the European Communities would offer the following brief comments:

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[222] Chassy, B., Hlywka, J.J.,. Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Re, E.B., Reid, J.E. (2004) Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology, Prepared by a Task Force of the ILSI International Food Biotechnology Committee. Comprehensive Reviews in Food Science and Food Safety 3: 35-104.
http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

[223] Monsanto (2000) Updated Molecular Characterization and Safety Assessment of Roundup Ready Soybean Event 40-3-2. Monsanto Co. St. Louis, 20 pp.
http://archive.food.gov.uk/pdf_files/acnfp/summary.pdf

609. The European Communities would refer to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following). In the present case, the notification contained no evidence of actual verification of the insert by sequencing. Note that it had been submitted on February 15th 2001, by which date sequencing had been routine for almost a decade.

610. The Health Council of the Netherlands in EC-95/At.8 (June 28th 2001) requested specifically sequencing of approx. 1000 bp of genomic DNA flanking the insert, as well as sequencing of the complete insert itself. It was not clear from the responses of the notifier (EC-95/At.10-12, February 2002-February 2003) if the full length insert had been characterised by sequencing of the transformed maize, or whether only the flanking sequences were verified from the GM maize, while the rest of the insert sequence was simply determined by sequencing the transforming plasmid. Furthermore, in the responses from the notifier only 271 bp of the 3'-flanking sequence was provided. The need for as much as 1000 bp was reasonable given the state-of-the-art concerning gene regulation in plants at the time the request was made

Question 52

Given the information before the Panel, including the application and the responses from Pioneer/Dow AgroSciences (referenced above), was information regarding the potential unintended expression of allergenic proteins requested by Gezondheistsraad (EC-95/At.15) necessary to ensure that conclusions of the safety assessment were valid?

General comments

611. As explained in the European Communities comments on Dr. Nutti's reply to question 43, Dr. Nutti's approach to issues of allergenicity does not take into account the state of the analysis of molecular characterisation of the product. That analysis, however, is necessary to comment on toxicology problems regarding allergenicity (and unintended effects). Dr. Nutti's conclusion ("as far as my knowledge goes") therefore, may be valid as regards general toxicity aspects of the CRY1F protein, but it does not apply to the actual considerations (related to molecular characterisation) that the lead CA had raised.

Detailed comments

612. As the CA clearly explains, the request for the further allergenicity assessment addresses problems of unintended molecular effects of the flanking regions, especially the potential fusion proteins formed by such effects.

613. This request is fully in line with modern standards of assessment of unintended effects in flanking regions. Thus Codex guideline CAC/GL 45-2003 states in its paragraph 31D:

> 31. Information should be provided on the DNA insertions into the plant genome; this should include:
>
> D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

while it also states in paragraph 5 of its Annex on the Assessment of Potential Allergenicity that:

> As there is no single test that can predict the likely human IgE response to oral exposure, the first step to characterize newly expressed proteins should be the comparison of the amino acid sequence and certain physicochemical characteristics of the newly expressed protein with those of established allergens in a weight of evidence approach.

614. The CA's request for additional information on putative fusion proteins was in line with these standards and therefore justified.

Question 53

Given the information before the Panel, including the application (EC-96/At.1-2), was information regarding molecular characterization, toxicity effects of unintended changes and compositional data requested by Gezondheitsraad (EC-96/At.7) necessary to ensure that conclusions of the safety assessment were valid?

General comments

615. Dr. Nutti comments on the aspects related to compositional analysis and toxicological analysis. As has already been pointed out before, Dr. Nutti's approach does not take into account the need for a thorough analysis of the molecular characterisation, which makes her conclusions incomplete or flawed. More specifically, her conclusion that a semi chronic toxicity study is not necessary must be dismissed on the basis of the available scientific evidence on the relevance of such studies.

616. Finally, as she has not commented on the specific requests for additional information on molecular charcterisation, the European Communities will offer brief comments.

Detailed comments

617. Given the publication year (2001) of the requested 90-day study on rats (Dudek, 2001, cited in EG96, At.2, p. 117), the applicant must have completed this report by the time the CA made its request for additional information (December 13th, 2001), *i.e.* this information could have been almost available by that time. These data should therefore have been taken into account, in line with paragraph 15 of the Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, which states that:

> Risk assessment should take into account *all available scientific data and information* derived from different testing procedures, provided that the procedures are scientifically sound and the parameters being measured are comparable. [Emphasis added]

In addition to this requirement, arguments for requesting a 90-day toxicity study on whole products of maize NK603 are provided below.

618. The rationale provided by CA for its request for a semi chronic toxicity study was that unintended, additional modifications might have taken place in maize NK603, including modifications that had already been describe by the applicant.

619. With regard to animal studies, the applicant had provided (a summary of) a nutritional study in chicken broilers fed whole products and acute toxicity in rodents with the purified transgenic

proteins EPSPS. It should be stressed here that the chicken broiler study is not a model for toxicology, but for nutrition (for example, see the discussion on this issue in Chassy et al., 2004).[224] During studies on broilers, the growth, bodyweight, feed consumption, and weight and composition of edible parts after slaughter are usually measured. Therefore, the toxicity animal studies had been limited to those on acute toxicity of purified EPSPS, and no toxicity studies with the whole food.

620. According to Dr. Nutti, the additionally requested 90-day toxicity study was not necessary, since compositional equivalence had been demonstrated and the intended modification (the EPSPS protein) had also been tested for potential toxicity and allergenicity. The Dutch CA, on the other hand, provided as rationale for its request for the 90-day study that it would provide additional reassurance of no unintended effects undesired effects (EC96, At.7).

621. Whole feed/food studies can be used in order to provide reassurance about the potential health consequences of unintended effects, such as those may have been caused by the insertion of additional DNA fragments, beyond the intended modifications (e.g. transgenic proteins) that have been tested for toxicity. In fact, other ways to detect unintended effects, such as the use of advanced analytical technologies called "profiling" or "metabolomics", are in development, but have not been validated yet for routine application in the safety assessment of GM foods.[225]

622. An example of animal feeding studies serving as additional reassurance of no adverse effects would be the case of Roundup Ready soybeans. It was discovered in 2000 that unintended insertions of DNA fragments had occurred in these soybeans, which had already been approved as a GM food in a number of countries, including the EU. The applicant (Monsanto) itself argued that these additional fragments could not pose a risk, since, among others, no adverse effects had been observed in a previously published peer-reviewed animal feeding study with whole feed products derived from Roundup Ready soybeans.[226]

623. The lead CA, therefore, was justified, in the absence of toxicity studies with whole foods, to seek additional reassurance for the innoxiousness of any unintended effects by asking for an animal toxicity experiment with the whole food. In addition, such a study (Dudek, 2001, At96, At.2, p.117)[227] had already been published by the applicant at the time of the request (or shortly thereafter, in 2001), and the report of this study could therefore have been requested for the sake of completeness of the safety evaluation.

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[224] Chassy, B., Hlywka, J.J.,. Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Re, E.B., Reid, J.E. (2004) Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology, Prepared by a Task Force of the ILSI International Food Biotechnology Committee. Comprehensive Reviews in Food Science and Food Safety 3: 35-104.
http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

[225] Chassy, B., Hlywka, J.J.,. Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Re, E.B., Reid, J.E. (2004) Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology, Prepared by a Task Force of the ILSI International Food Biotechnology Committee. Comprehensive Reviews in Food Science and Food Safety 3: 35-104.
http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

[226] Monsanto (2000) Updated Molecular Characterization and Safety Assessment of Roundup Ready Soybean Event 40-3-2. Monsanto Co. St. Louis, 20 pp.

[227] Dudek, B.R., 2001. Amendment 1 to final report. 13 Week Feeding Study in Rats with Grain from Roundup Ready® Corn (NK603) Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Rodent Diet #5002. Monsanto Technical Report MSL 17555 (Amendment to MSL 17423), St Louis, Missouri

624. Finally, as regards the CA's request for additional information on molecular characterisation (on which Dr. Nutti has not commented), the European Communities would offer the following brief comments:

625. The European Communities refers to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following). In the present case, it should be noted that the request is dated December 13th 2001, i.e. almost one year after the publication of the paper on GTS 40-3-2 (Windels et al., 2001).[228] Thus, the scientific basis for requesting full sequence data of approx. 1000 bp flanking each side of the insert is justified, also on the basis of state-of-the-art knowledge and technology. Clearly, Southern blot analysis was insufficient as evidence by this date. Therefore, the requested data was necessary to ensure that conclusions of the safety assessment were valid.

Question 53bis

Given the information before the Panel, including the first whole food study and agronomic performance tests (EC 96/At.2, pp. 102-103), was a second animal whole food study (EC 96/At. 7) necessary or useful to identify potential adverse effects, including those associated with small random DNA insertions, that had not been previously identified?

General comments

626. The question refers to the lead CA's request for a semi chronic toxicity study (question 2 in EC-96/At.2), which was already covered by and addressed in question 53. Dr. Nutti's comments in essence are identical to her comments in question 53 on that issue. Therefore, the European Communities refers to its above under question 53.

Question 54

Given the information before the Panel, including the application, was information requested by Italy (EC-96/At.9) necessary to ensure that conclusions of the safety assessment were valid?

General comments

627. Dr. Nutti comments on one specific request the Italian CA made, namely a request for further animal studies. Her conclusion that such data would not be necessary has to be dismissed on the same grounds as have been put forward in question 40 on the issue of feeding studies with farm animals with physiologies different from that of the broiler chicken Therefore, the European Communities refers to its above comment under question 40.

Question 57

Given the information before the Panel, including the application (EC-101/At.1-3) and the additional information provided by Pioneer (EC-101/At13), was additional information molecular characterization, field trials, secondary plant metabolites, and toxicological tests requested by the Netherlands (EC-101At.14) necessary to ensure that conclusions of the safety assessment were valid?

[228] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

General comments

628. Dr. Nutti, Dr. Andow and Dr. Squire comment on this question. Dr. Squire generally confirms the CA's request as "consistent with the type of information indicated in the *Codex Guidelines for the conduct of food assessment of foods derived from recombinant DNA plants*." Dr. Nutti and Dr. Andow provide specific comments on questions 2, 3 and 4 raised by the Dutch CA. On questions 2 and 3 the two experts come to opposite conclusions. The European Communities agrees with Dr. Andow's conclusions. On question 4, the debate on the necessity of certain animal toxicity studies on hybrids is identical to the one in question 44. The European Communities, therefore, refers to its comments in question 44.

Detailed comments

629. On the issue of data on herbicide treatment Dr. Nutti concludes that the required information is not relevant to ensure that conclusions of the safety assessment were valid. In that she is contradicted by Dr. Andow who concludes that "there is a scientific justification for requiring comparison of T25 with glufosinate, T25 without glufosinate and maize without T25 and without glufosinate." The European Communities agrees with Dr. Andow. Dr. Andow adds that he believes "that there is a legitimate scientific debate as to whether all three are necessary to ensure that conclusions of the safety assessment were valid." The fact that there is a scientific debate does not call into question the CA's request (which Dr. Andow himself recognises as scientifically justified). For the reasons already set out in its earlier comments, eg on question 49, the European Communities sides with the view that the CA has taken, namely that the comparison of all three sets of data, which the applicant indicated to be readily available (EC-101, At.13, A2, p.2), is necessary.

630. On the request for data on secondary compounds Dr. Nutti is of the opinion "that information required is not relevant to ensure that conclusions of the safety assessment were valid, as the compositional information supplied before leads to the conclusion that the product was substantial equivalent." She adds that "in this case, we have a product that is a conventional cross of two GM corns, but this product has not been submitted to a new genetic modification, so the possibility of fortuitous changes in the plant seems to be vanishing small, if exists." In this assessment, Dr. Nutti is contradicted by Dr. Andow who finds that "There is a legitimate concern that there are additional transgenes incorporated into the T25 and Mon810 lines that could be expressing additional but unknown gene products, and that elude detection by present molecular methods. In addition, it is also possible that the main gene products from T25 or Mon810 could interact with plant metabolism, changing the composition of the plant." While Dr. Andow also speaks of a "small likelihood" of this happening, he finds that "it is difficult to argue how small."

631. The European Communities agrees with Dr. Andow's assessment. While the issue of conventional crosses of GM lines is not specifically addressed by the Codex guideline, it does so for the issue of unintended effects, for example in paragraph 15 (Unintended effects):

> Unintended effects may also result in the formation of new or changed patterns of metabolites"

632. Dr. Nutti states that compositional analysis would already have led to the conclusion that the conventional cross is substantially equivalent. This compositional analysis was, however, "targeted", *i.e.* specific compounds have been measured. Any changes in compounds that have not been measured would therefore remain unnoticed.

633. Dr. Nutti also asserts that additional unintended effects are hardly possible in conventional crosses of GM lines. For example, the introduced GM traits from the particular parent plant may interact with each other within the conventional cross of GM lines.

634. One example is that of "gene silencing" (inactivation of gene function) by bringing together genes with similar sequences from different parents within the same plant (as observed, for example, by Charrier et al. (2000) and De Wilde et al. (2001)).[229]

635. Based upon the considerations above, including the fact that unintended effects in conventional crosses of GM lines cannot be excluded on beforehand, the request of the lead CA was relevant from the point of view of safety of the cross of two GM crops and any secondary metabolites that have been changed as an unintended side-effect of the combination of genetic modifications.

636. On the request for additional toxicity testing, both Dr. Nutti and Dr. Andow take the view that there is no need for such tests albeit for differing reasons. Both have discussed their position in more detail in questions 44 and 44bis. There are valid arguments to refute either's position. The European Communities has discussed those in question 44 and 44bis, therefore, refers back to its comments therein.

637. Finally, as regards the CA's request for additional information on molecular characterisation (on which no expert has commented), the European Communities would offer the following brief comments:

638. The European Communities refers to the internationally agreed principles regarding the necessity of detailed molecular characterisation in a thorough safety assessment (see section III on general and methodological issues, and the expert replies on Q 9, 111 and following) as well as to its comments in questions 44 to 44ter.

639. Generally, stacked hybrids will require a separate molecular characterisation and complete risk assessment, and can not only rely on evaluation of the parental GM events. Furthermore, the request from the Netherlands (EC-101/At.14, dated April 23rd 2001) was necessary to ensure that conclusions of the safety assessment were valid. By this date DNA sequencing had been applied routinely for characterisation for almost a decade. Furthermore, the Windels et al. (2001)[230] paper was published a few months prior to the request. This paper clearly demonstrated the relevance of detailed sequence information including the flanking sequences.

Question 58

Given the information before the Panel, including the application (EC-102At.1-20) and the information provided by Monsanto/Novartis (EC-102/At.22, 26 and 27-30), was additional information regarding food safety assessment of derived proteins requested by the Netherlands (EC-102/At.32) necessary to ensure that conclusions of the safety assessment were valid? Did the food safety assessments provided by the applicant follow the Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants.

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[229] Charrier, B., Scollan, C., Ross,S., Zubko, E., Meyer, P. (2000) Co-silencing of homologous transgenes in tobacco. Molecular Breeding 6: 407-419.

De Wilde, C., Podevin, N., Windels, P., Depicker, A. (2001) Silencing of antibody genes in plants with single-copy transgene inserts as a result of gene dosage effects. Molecular Genetics and Genomics 265: 647-653.

[230] Windels P., Tavernier I., Depicker A., Van Bockstaele E. & De Loose M. (2001). Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

General comments

640. Dr. Nutti concludes that "there was no need for requesting a semi chronic toxicity study in mice or rats using the edible parts of the sugar beet in order to rule out possible undesired effects of additional unidentified changes." The request for a semi chronic toxicity study had been one of the CA's requests. The European Communities disagrees with Dr. Nutti's conclusion on the grounds already set out in questions 44, 51, 53, and 57. The CA had also raised specific concerns on the potential presence of a certain fusion protein (protein 34550), a concern which Dr. Nutti does not specifically address in her comments, presumably because that question pertains to issues of molecular characterization, which would be outside the scope of Dr. Nutti's comments.

VI. THE PANEL'S QUESTIONS ON ISSUE 2 ("SAFEGUARD MEASURES") (NOS 59 TO 95)

A. INTRODUCTORY REMARKS

641. The Panel's questions 59 to 95 concern the Member State safeguard measures. Generally, for each measure, there are three questions, concerning: the sufficiency of scientific evidence; the risk assessment, and its relation to international guidelines; and the justification for provisional measures, in the light of other available risk management options.

642. The European Communities would like to make the following general comments.

643. First, the European Communities understands that what the Panel's question aims to elucidate is the issue of the point at which the information or scientific evidence is sufficient for the authority to decide *definitively* whether or not to approve the product; and the issue of whether or not that point had been reached in the specific circumstances of the Member State safeguard measures.

644. Second, in science as in law, it may be problematic to prove a negative, especially prospectively. That is, it may be problematic for a scientist to certify definitively that mass commercialisation of a new product will have no adverse effects on human health. In making a risk assessment, it is likely that the scientist may have to place the product and the issues associated with it in context. The scientist may need to get some kind of "helicopter vision" on the problem. The new product may be just one feature of the scientific landscape – and the scientist may need to consider the landscape as a whole before taking a view on the relative safety of the product. In these circumstances, the absence of information or evidence about the context may be just as significant as the absence of information or evidence about the product itself.

645. Third, the Appellate Body has made it clear that the concept of "sufficiency" is a relative one – the scientific information must be sufficient for a specific purpose, that is, for the purposes of deciding whether or not to approve the product definitively, taking into account the legislator's chosen level of protection. The higher the chosen level of protection, the more scientific evidence may be necessary before the evidence is sufficient to make a definitive decision, and the longer the period of time and the greater the resources that may reasonably be required.

646. Fourth, the evaluation will also be a function of the information or scientific evidence available: the less information or scientific information available, the less detailed the evaluation may be.

647. Fifth, it may well be that the available information or scientific evidence and the evaluation or assessment are insufficient to support a definitive refusal to authorise: but that it is still sufficient to

support a provisional measure, at least if the conditions of Article 5.7 of the *SPS Agreement* are complied with. This is the position with regard to the Member State safeguard measures.

648. Sixth, as the European Communities has already explained in its submissions, to fairly understand why the Member States acted as they did it is not sufficient to look only at the text of the provisional measures themselves (which is often relatively succinct), and the notification to the Commission. It is also necessary to consider the history of the discussions, including the discussions at Community level, in which all the Member States participated. From this perspective, it is unnecessary to distinguish between safeguard measures adopted by different Member States, except insofar as there are additional considerations specific to that Member States (such as climate, agricultural structures and practices, flora, fauna, etc.). In order to avoid unnecessary repetition, the European Communities will, in these comments, refer back to the more general observations that have already been made.

649. Seventh, the European Communities would note that the responses to the three types of question often overlap to a significant extent. In such circumstances, where appropriate, the European Communities will comment in general terms in relation to each product for which a safeguard measure has been adopted.

650. With these general remarks in mind, the European Communities will now comment on the expert advice with respect to each of the products.

B. QUESTIONS 59 THROUGH 65

Question 59

Given the information before the Panel, including the evaluations undertaken by the UK (EC-161/At.1), the Scientific Committee on Plants in May 1998 and May 1999 (CDA-35-A and CDA-69, respectively), and the European Commission in its Decision of February 1996 (CDA-62), as well as the information submitted by ***France*** *with respect to its safeguard measure (EC -161/At. 3 to 11; CDA-68, CDA-70, CDA-71), is there any reason to believe that the scientific evidence available to France in November 1998 and July 2001 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from the growing for seed production of oilseed rape MS1 x RF1? If so, what scientific evidence do you believe was insufficient?*

> *(a) If the evidence was not sufficient in November 1998 and/or in July 2001, was there sufficient evidence available to France in August 2003 to permit it to undertake a more objective assessment of potential risks to human, plant and animal health, and the environment from the growing for seed production of oilseed rape MS1 x RF1? If not, what scientific evidence do you believe was insufficient?*

651. The European Communities would draw the Panel's attention to the fact that, in response to this question, Dr. Squire notes that: "The EC SPC and the information submitted by France therefore agreed on the nature of this potential problem, but differed on its importance or extent"; and that "There was insufficient knowledge at that time to be able to predict accurately, for a country such as France, what the rates of spread and cross pollination would be (GM to non-GM) if a large part of the

rapeseed areas were GM". Dr. Squire further notes that even at the end of 2003 no conclusions can be drawn and that work is still in progress, citing to specific references[231].

652. Furthermore, Dr. Snow advises as follows:

> Given that, 1) the SCP identified concerns about the spread of herbicide resistance to volunteer weeds, even though they considered this to be more of an agronomic problem than an "environmental" one; 2) the SCP underestimated the extent of gene flow that is to be expected to other oilseed rape crops, volunteer weeds, and weedy Brassica rapa; and 3) the SCP recommended that the introduction of herbicide-tolerant crops should be accompanied by "ii) a monitoring programme with an agreed design and implementation plan to detect the occurrence and the establishment of herbicide-tolerant volunteers and weeds under field conditions in the EU;" I conclude that France had valid reasons to follow the advice of its Biomolecular Engineering Committee to carry out more research to "supplement existing scientific knowledge and validate methods for managing the cultivation of genetically modified oilseed rape." Scientists in France were well aware of the fact that transgenes would be dispersed by means of pollen and seeds, making it difficult to design management plans that would prevent problems in the future. These problems could be compounded if transgenes for glyphosate tolerance were also approved in oilseed rape and other crops, as I explain elsewhere. One could argue that future events are not relevant to this isolated product, but glyphosate-tolerant oilseed rape has also been proposed for commercialization in Europe."[232]

653. With regard to environmental issues, Dr. Nutti gives no advice[233]. With regard to human, plant and animal health, Dr. Nutti expresses the opinion that the information submitted was "adequate"; that there was "no evidence to indicate" that the product was "likely" to cause adverse effects; and that there was "no scientific evidence" on the basis of which France could "ask for the moratorium"[234].

654. In the opinion of the European Communities, Dr. Nutti's advice on this point is of little if any assistance in resolving the relevant issue. First, Dr. Nutti appears to have considered whether or not the science could have justified a definitive decision not to approve the product – concluding that this is not so. However, that is not the pertinent issue. The pertinent issue is whether or not the science could justify a provisional measure – a matter that Dr. Nutti does not consider at all. Second, the question for an authority in such a situation is not whether or not the information is merely "adequate" – the standard applied by Dr. Nutti. Rather, the question is whether or not the science is sufficient for a definitive decision to be taken. Depending on the acceptable level of risk chosen by the legislator "adequate" may well fall far short of "sufficient". Third, Dr. Nutti appears to have considered whether or not the science established that the product "is *likely* to cause adverse effects". But that is not the applicable standard. In order to provisionally exclude a product, an authority is not required to show that the product "is likely" to cause adverse effects. Rather, the authority must comply with the conditions set out in Article 5.7 of the *SPS Agreement*, which set out a different standard. Fourth, Dr. Nutti's conclusions are based on the alleged *absence of evidence* to indicate likely adverse effects. That is an erroneous expression of the burden of proof issue.

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[231] Squire, pages 14 and 15.
[232] Snow, page 21.
[233] Nutti, page 43.
[234] Nutti, page 43.

655. Having regard to these fundamental erroneous assumptions, the European Communities considers Dr. Nutti's advice to be of no value in resolving the issues before the Panel.

656. The European Communities concludes that, on the environmental issue, the expert advice given unanimously supports the position of the EC; and that on the issue of human, plant and animal health, no expert advice has been given capable of calling into question the lawfulness of the view that, for France's purposes, the scientific evidence was insufficient to adopt a definitive decision.

Question 60

With reference to the definition of a risk assessment in the SPS Agreement (see Background above), to what extent does the scientific evidence and other documentation submitted by France evaluate the relevant risks to human, plant or animal health, and the environment from the growing of oilseed rape MS1 x RF1 for seed production?

> *(a) How does the scientific evidence and other documentation submitted by France compare with the relevant international guidelines for risk assessment and analysis identified above?*

657. The European Communities would draw the attention of the Panel to the observation of Dr. Squire: "It could be argued that France's position was compatible with the tone of the SPS Agreement, Annex A, paragraph 4 (including economic as well as biological consequences) and compatible also with ISPM-11 Annex 3 on 'Determining the potential for a LMO to be a pest'."[235]

658. The European Communities would also refer to Dr. Snow: "To the extent that these concerns are justified in the context of invoking "safeguard" measures (which I consider to be a legal question), I conclude that France had valid reasons for deciding that additional scientific research was needed."[236]

659. With regard to Dr. Nutti, the comments outlined above also apply, *mutatis mutandis*.

660. Essentially, the conclusion with respect to question 60 follows inevitably from the conclusion with respect to question 59. Once the insufficiency of the scientific evidence is established, it follows that, even on the Complainants' (erroneous) reasoning, there can no longer be any possible controversy about the provisional nature of the measure. The relevant provision being Article 5.7 of the *SPS Agreement*, what is required is that the authority act "on the basis of available pertinent information", making an assessment appropriate to the circumstances. Thus, the extent of the evaluation will always be a function of the available information and science – a view that is also reflected in the relevant international guidelines.

Question 61

Does the scientific evidence and other information submitted by France support the adoption of a temporary prohibition on the growing of oilseed rape MS1 x RF1 for seed production? In light of any potential risks identified by France, what other risk management options were available in November 1998 and/or July 2001? What other risk management options are now available?

[235] Squire, page 15.
[236] Snow, page 22.

661. The European Communities notes that, according to Dr. Squire, "The risk management options were and are similar to those indicated generally for oilseed rape … The risk of spread and cross-pollination can be reduced by such measures but not eliminated"[237].

662. Dr. Snow observes:

> I do not have enough information to answer this question. If the question refers to seed production on a very small scale, a temporary prohibition presumably would be less urgent and possibly unnecessary. If the plants are grown on a fairly large scale, for example on 1,000 hectares per year, the issues at hand are similar to those for the previous questions.
>
> The basic concern is that herbicide resistance genes would spread in conjunction with wider use of this herbicide over time, and that other types of herbicide resistance genes might also be approved in the future (e.g., glyphosate resistance), thereby compounding the problem. One could argue that future events are not relevant to this isolated product, but glyphosate-tolerant oilseed rape has also been proposed for commercialization in Europe.[238]

663. According to Dr. Andow:

> The adoption of a temporary prohibition can be justified on the basis of the scientific evidence and other information submitted by France.
>
> Several other risk management options could also have been justified in November 1998 and July 2001. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. In 1998 and 2001 mitigation and tolerance strategies were probably inappropriate. Here I consider only the risk that France expressed in its original decision to impose a temporary prohibition. One risk avoidance strategy would have been to allow limited planting in a restricted region. In the first year, this could have been at a scale of a large field trial, and build up from there. This would allow determination of scale effects. Another strategy is outlined in paragraph 3.19.
>
> Today several alternative risk management options are available. In addition to risk avoidance as in the previous paragraph, risk mitigation strategies may also be possible to control HT volunteers and wild species. It would appear that France is now convinced that if HT volunteers or wild species occur that they can be detected rapidly enough and eliminated.[239]

664. The position with regard to Dr. Nutti has already been outlined above.

665. The European Communities observes that concerns raised by France about rapeseed applications in 1998 focused on the impact of cropping systems and agricultural practices on the spread and establishment of HT plants, *via* volunteers or hybrids with wild relatives. Although scientific knowledge was already available on pollen flow and out-crossing rate to wild relatives, several issues remained open:

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[237] Squire, page 15.
[238] Snow, pages 23 and 24.
[239] Andow, page 84.

- Information available on gene flow (e.g. long distance gene flow) was obtained from small-scale experiments and was difficult to extrapolate to large-scale dissemination of GM crops;
- The effect of the various cropping systems and agricultural practices one may find in European agriculture and which would exert a selection pressure on HT plants was not assessed;
- Inter-specific hybrids with very common wild relatives Raphanus (wild mustard) in French cropping systems had been recently established[240]);
- The precise contribution to gene flow of feral plants which are present in non-cultivated areas was clearly not available.

666. This meant that, depending on the legislator's chosen level of protection, it was possible that the overall ecological and economic balance of growing HT rapeseed varieties could not be assessed. This statement was clearly applicable to crop production. As to the seed production, even if the scale is lower than for crop production, the acreage of rapeseed production was 4,314 ha in 2004 in France. Even if it was unlikely that seed production of *MS1 x RF1* would cover all this acreage, the figure of 1,000 ha mentioned by Dr. Snow is realistic. As seed production is performed in various regions, this could be considered as a significant dissemination (at least much higher than field experiments).

667. Furthermore, if seeds are produced, it would be likely that crop production would occur later and it seems reasonable to anticipate larger scale dissemination (in fact, an application for cultivation was already submitted before 1998). The same kind of argument is considered relevant for taking into account the impact of herbicides on HT crops even if the herbicide is not yet approved for commercialisation.

668. With respect to imports, the exposure may be lower as no intended dissemination through cultivation would normally occur. Nevertheless, spillage from transport or during handling of imported seeds can occur[241]. Even if the potential for spread and establishment could be considered quite low (especially in the case of glufosinate tolerance), there were uncertainties about out-crossing to wild relatives, spread and establishment of feral plants (see above) while the detection of such events in the environment was not yet available.

669. Wild relatives that outcross with B. napus are present in various regions: wild radish (raphanus) and cabbage (B. oleracea) whose European coastal zone is a centre of diversity.

670. The European Communities would also note that if imported seeds entered into cultivated fields (directly through seed dispersal or by pollen flow from feral plants to cultivars), their development could then be maintained in the case of farm-saved seeds (more than 30 % of the French cultivars).

671. With regard to the assertion that imports would concern spring rapeseed and, consequently, lead to a reduced risk of establishment, the European Communities observes that, in fact, spring cultivars are mainly characterized by a shorter cycle, but their frost tolerance, although lower than winter types, does allow them to survive winters in many parts of Europe.

[240] Chèvre AM, Eber F, Baranger A, Renard M. 1997. Gene flow from transgenic crops. Nature 389: 924.

[241] Crawley et al, 1993.

672. Since 1998, various studies have been carried out to address the specific concerns outlined above and the initial scientific statement was updated in 2001 and 2003.[242] Long distance gene flow is now better established even if the precise role of insects in cross-pollination is still under investigation. The potential for long persistence of HT plants has been demonstrated through the post-release monitoring of HT field trials: in one location site, and six years after a HT field trial, GMHT volunteers still accounted for almost 2% of the plants in a non-GM rapeseed field; and this occurred without any selection pressure as the glufosinate had not been used in that area[243]. Such a phenomenon was only observed in one of several locations. However, this, once again, means that it was legitimate, as a matter of WTO law, including under the *SPS Agreement*, for France to take into account the great diversity of agro-ecosystems.

673. Gene flow models have been designed to assess the effect of cropping systems and of management practices leading to a better predictive capacity (see question 6). In that context, it has been confirmed that, under some agricultural practices, feral plants could highly contribute to the spread and establishment of HT crops[244].

674. In the light of these observations, the European Communities again concludes that the weight of expert advice to the Panel overwhelmingly supports the position of the European Communities in these panel proceedings with regard to the French safeguard measure for *MS1 x RF1*.

C. TOPAS 19/2 (NOTIFICATION C/UK/95/M5/1) (FRANCE AND GREECE) (QUESTIONS 66, 67 AND 68)

675. Essentially, many of the issues and expert comments with regard to *Topas 19/2* are very similar to those regarding *MS1 x RF1*, except that *Topas 19/2* was approved for seed processing rather than cultivation. The European Communities will not therefore re-iterate the questions or the experts' comments, it being sufficient to observe that, whilst the risks may not have been quite as acute, they were nevertheless there. And in this context it should not be forgotten that the relevant question is whether or not the Member State authorities, applying their chosen level of protection, were entitled to adopt a provisional measure on the basis of available pertinent information.

676. The European Communities would in particular note that Dr. Andow confirms that, at least in 1998, an authority would have been entitled to take the position that the science was insufficient to take a definitive view. Areas of concern included molecular characterisation (a particularly fundamental issue) and co-existence. Furthermore, the Member States could reasonably have taken the view that the risk management measures proposed by the SCP were not supported sufficiently by science. Dr. Andow observes that: "The scientific evidence in 1998 on the agricultural practices necessary to eliminate HT volunteers and wild species resulting from spillage may not have been sufficient to complete an accurate assessment of risk management practices."[245]

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[242] Opinion of the French Biomolecular Engineering Committee (BEC), 02-2001; BEC Opinion 02-2004.

[243] CETIOM, internal report.

[244] Bock, A.-K., Lheureux K,. Libeau-Dulos M. Nilsagard H., Rodriguez-Cerezo E., 2002. "Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture", Technical Report Series of the Joint Research Center of the European Commission, EUR 20394 EN., 133p.. Angevin F., Colbach N., Meynard, J. M., 2003. Coexistence of Rapeseed Varieties in Time and Space: Using GeneSys Model to Adapt Cropping Systems. 11th International Rapeseed Congress, Copenhagen, 6-10 July 2003, pp 732-734. Colbach N., Angevin F., Meynard J. M., Messéan A., 2004 Using the GeneSys model quantifying the effect of cropping systems on gene escape from GM rape varieties to evaluate and design cropping systems. OCL, 11: 11-20.

[245] Andow, page 86.

677. The European Communities would further note that, at least at the time these measures were adopted, gene/pollen flow uncertainties and coexistence were still a significant and relevant area of scientific uncertainty, in particular for multiple undesired HT gene stacking in oilseed.

678. There was also a significant outstanding issue at the time regarding possible seed escapes from seed imports and seed dormancy (in particular in the context of the particular seed properties of oilseed (size, longevity, etc...), and the well known risk of feral escapes during transport from the field to the crop or the processing plant. These risks had to be considered also in the context of the then limited availability of proper management tools (including pollen flow understanding and surveillance and monitoring).

679. Even today, the risk arising from possible seed escapes must still be placed in the context of the climate in the region of import, including where spring rape is not normally agronomically grown, and where winter frost may not be prevalent. There may be significant differences, for example, between Mediterranean ports and Scotland. The risk must also be placed in the context of possible potential "systemic" HT risks, if several different HT characteristics become present in oilseed rapes from undesired pollen sources, and giving due consideration to HT oilseed in the context of agro management issues, where there are several different HT crops in rotation, which in turn could be considered to gradually increase prevalence of glufosinate use.

680. In this context, if there is long seed dormancy, and if there had been a general increase over the years of the use of HT crops, potential oilseed escapes, even limited, may reasonably have been perceived then to potentially become a problem later, given also that tools were not yet identified at the time which would have allowed appropriate management or reversal of the situation, if it had arisen.

681. A related aspect of the problem is that once cultivation of some HT crops has been authorised, a decision has also been implicitly taken in principle regarding future HT crops – even before a full risk assessment may have been completed. This would, for example, be the case when it comes to authorising glufosinate wheat in the context of authorised glufosinate oilseed, sugar beet and maize, even if the issues regarding oilseed at that time would have been limited to escapes from imports.

682. With regard to Greece, the European Communities would also note that Dr. Andow does consider that, with reference to the *SPS Agreement*, the Greek case did raise relevant risks to plant health. Dr. Andow also considers that the "potential pest" concept derived from ISPM should be considered, and that such argument holds for this particular case because Greece did identify a potential risk, and because other potential risks could be identified. He further states that, for those risks within the scope of ISPM 11, the risk assessment process was consistent with ISPM 11. In addition, he notes that the science, documentation and reasoning of Greece are consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Specifically, Annex III, Section 8(f), which states: "Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment."[246]

683. The Mediterranean region (including Greece) is a centre of diversity for wild crucifer species, and gene flow from GM brassica crops to these wild species is therefore an issue for consideration in a risk assessment in Greece (Chèvre et al 2004). If escaped Topas 19/2 plants establish, then there is a possibility of gene flow to these related wild crucifer species which occur in Greece particularly in

[246] Andow, pages 89 to 91.

some coastal areas. There was (and still is) uncertainty about gene flow to wild relatives of the GM crop and possible consequences for rare or uncommon species.

D. MAIZE BT-176 (NOTIFICATION C/F/11-03) (AUSTRIA, GERMANY, LUXEMBOURG) (QUESTIONS 69 TO 77)

1. Introductory remarks

684. The European Communities considers that Dr. Nutti's response to question 69 is seriously flawed.

685. First, as the European Communities has explained, it considers that question 69 is aimed at ascertaining whether or not the science was sufficient for an authority to adopt a *definitive* decision on whether or not to approve the product. Dr. Nutti's conclusion does not address this issue at all. Rather, Dr. Nutti concludes only that the science was sufficient for an appropriate assessment. That is self-evident, the information or science being sufficient *for Austria* to adopt a *provisional* measure, based on an appropriate assessment. Dr. Nutti's conclusion therefore makes no contribution at all towards resolving the dispute between the parties.

686. Second, Dr. Nutti offers no detailed statement of fact nor developed line of scientific reasoning to support her conclusion, confining herself to references to certain opinions from certain scientific committees. She fails completely to address the issue from the perspective of the specific legislator's chosen level of protection.

687. Third, she extends her conclusion to environmental issues, even though she repeatedly states that environmental issues are not her field, and that she cannot therefore offer any advice in that respect.

688. The European Communities considers that question 70 is designed to assist the Panel in determining whether or not, underpinning Austria's safeguard measure, there was an appropriate assessment or evaluation. The European Communities considers that there was, and has pointed in this respect not only to the additional documents produced by Austria, but also to the existing risk assessments. Dr. Nutti confirms this – re-iterating what the European Communities has already referred to, namely the adequacy of the relevant risk assessments. Dr. Nutti thus confirms the position of the EC.

689. With respect to question 71, Dr. Nutti states that she considers that there is no support for a temporary prohibition, but offers no explanation for this assertion. The assertion is, in any event, profoundly perplexing, given that it is uncontroversial that the initial temporary restrictions imposed by the general legislation, which are not challenged in these proceedings, are not inconsistent with the WTO Agreements. Placed in this context, Dr. Nutti's unqualified and unexplained comments offer no assistance in the resolution of the present dispute. Similar comments apply with regard to Dr. Nutti's assertions concerning allergenicity and toxicology, insofar as no consideration is given to the question of whether or not Austria, acting provisionally, based itself on available pertinent information.

690. Similar comments apply with regard to Dr. Nutti's comments concerning Germany and Luxembourg.

2. Anti-biotic resistance

691. On the question of anti-biotic resistance, the European Communities refers to its comments to questions 1 and 2 above.

3. Non-target organisms

692. On the question of non-target organisms, apart from the submissions that it has already made, and taking into account the general comments that it has already submitted in these observations, the European Communities would like to make the following additional points. The European Communities considers that it is now clear that Bt toxin could accumulate in Bt-resistant herbivores (e.g. caterpillars which are able to ingest the Bt toxin and thus accumulate it and/or its metabolites without dying), and so pass the Bt toxin and/or its metabolites to organisms higher up the food web (e.g. to predators and parasitoids which feed on Bt-resistant herbivores). This point involving multi-trophic interactions[247] was not dealt with by the SCP in its analysis of risks to non-target organisms in the environment, at least not in a manner reflecting the specific legislator's concerns.

693. Another issue similarly not considered by the SCP in a manner reflecting the specific legislator's chosen level of protection is the point that any increase in Bt-resistant herbivores (target or non-target species) will increase the need for additional pesticide applications to control them. This 'knock on' effect, arising from the need to control Bt-resistant pests using additional pesticides could add additional environmental risk to local biodiversity (i.e. there is the potential for adversely affecting natural enemies, depending which pesticides were applied), spray operators (pesticide handling and exposure risks) and consumers (pesticide residue risks).

694. On economic grounds (cost: benefit) the requirement of additional pesticides on Bt corn 176 to control any Bt-resistant insects could negate any initial economic advantage of using GM crop event 176 (i.e. the savings on pesticide application could be lost and environmental harm could be increased).

695. The European Communities disagrees that the use of event 176 'and any progeny derived from crosses with traditional varieties' should automatically be considered unproblematic, taking into account the specific legislator's chosen level of protection. The genetic background of each parent as well as any unintended genetic changes during transformation and back-crossing are likely to introduce changes in plant metabolism and gene expression that can affect efficacy of the Bt toxin (important in the high dose / refuge strategy for insect resistance management) and nutritional value, *via* the potential for altered primary and/or secondary plant metabolites[248]. Such risks are mitigated (reduced) in plant breeding by several generations of back-crossing and careful selection of 'normal' crop phenotypes under a range of appropriate environments - taking several years, normally.

696. The European Communities agrees with Dr. Andow's opinions on the likelihood of Bt toxin entering the soil ecosystem (via plant debris and/or root exudates) and being more (not less) likely to cause harm to soil organisms (e.g. collembolan that consume plant remains in the soil) than residues from Bt sprays applied to foliage, where UV breaks down Bt toxin in foliar sprays quickly. The publication by Zwahlen et al. (2003) on earthworms was apparently criticised by EFSA in July 2004 as not being conclusive and definitive. The EFSA also made comparisons between growing Bt crops with risks from using Bt sprays (the latter are known to be UV unstable, contain different toxins, only present on leaf surfaces etc) that have been subsequently criticised. The cited criticisms by EFSA should at least have required that further follow-on scientific investigations were performed (precautionary approach after some evidence of adverse effects to an important soil NT organism), not that the scientific evidence should be dismissed and the potential risk to earthworms ignored. The EFSA also appeared to discount linked evidence that in GM maize lines lignin content was

[247] Groot & Dicke 2002; Plant Journal 31(4): 387-406.

[248] Haslberger 2001; Birch et al 2001; Ann Appl Biol 140: 143-149. See also the comments on the responses to question 3 for further details and citations.

unintentionally altered, leaving open possible long term effects on GM crop decomposition and nutrient cycling[249].

697. The European Communities agrees with Dr. Andow that by 2003 some, but not all, scientific uncertainties had been resolved. In particular, not all the most regionally important non-target species that could be exposed to Bt toxin *via* consumption of pollen from event 176 had been identified in Austria or other EC member states. Also, the risk of gene flow to non-GM varieties was still considered a valid risk by Austria in 2004 – an observation with which the European Communities would agree. The risks of pests developing resistance to Bt toxin, of adverse effects on regionally important non-target organisms and of gene flow to conventional corn, could all potentially be managed by Member States. Once sufficient region-specific research had been conducted in relevant Member States. The methods now being developed (e.g. GMO Guidelines project) may now (in 2005) assist Austria in developing suitable risk assessment and risk management strategies, but this would take some time to conclude and verify in each relevant Member State.

698. The EU project Bt-BioNoTa reported unintended effects of Cry1Ab proteins and Bt corn/cotton leafworm/green lacewing tri-trophic interactions[250]. These results have still to be published but add to already known uncertainties about effects of Bt crops and Bt toxins on NT organisms.

699. Specifically regarding question 69, Dr. Andow's comments that prior to 1997 impacts on non-target organisms and the risk of resistance had not been considered effectively are correct. There are at least two important issues here. First, the difference between the situation as it was generally understood in 1997 compared with 2003. Dr. Andow correctly points out that attitudes changed between these two dates. However, a more fundamental change was the increase in both the quantity and quality of scientific data on NTO effects. By 2003, better designed, more field-based studies had been conducted. Examples include the extensive studies on Monarchs by Hellmich, Sears and others published in 2001. These data models have been further refined[251] and clearly show that effects exist, even if they are small.

700. The second issue is the agricultural context. There are undoubtedly effects on at least some non-target organisms[252]. It may reasonably be anticipated that as more species are tested, more effects will be found. This supports the view it is very difficult to be sure that all appropriate NTO effects have been tested for.

701. A second difficulty which hampers interpretation of the evidence is that findings relating to NTO effects are often conflicting and may be highly specific to particular GM lines. For example there was early concern that lacewings would be affected by Bt[253] but more recent work has contradicted this[254]. Similarly with Collembola, there are at least 4 studies – 3 of which show no effect and 1 that has suggested the small effects observed are very specific to the particular type of cry1 product.

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[249] Birch et al., 2004, in Biodiversity and non-target impacts: a case study of Bt maize in Kenya, pp 117-188, CABI Publishing , UK.

[250] Scientific presentation by Ruud de Maagd at international scientific conference in Ascona, Switzerland, Sept 2003 – 'Biodiversity Implications of GM Plants'.

[251] E.g. Dively et al 2004.

[252] E.g. the examples mentioned by Dr. Andow, indirect effects on parasitoid abundance as in the Steffey et al. 2004 study and many others.

[253] Hillbeck et al.

[254] Romeis 2004.

702. The discussion about soil organisms nicely illustrates the difficulties in terms of sufficiency and NTO effects. It is a reasonable and lawful position to say that no Bt crops can be planted until there is information on all potential non-target organisms in the soil, particularly given that scientists do not know much about most of the organisms in the soil (they cannot be reared and it is not known what they feed on).

703. Another issue raised by Dr. Squire is that there is almost no information on the impact of non-GM Bt preparations, which is a real concern if this is suggested as a comparator in monitoring schemes. The comments of Dr. Andow relating to the temporary nature of Bt sprays must be seen in the light of the fact that there has been almost no monitoring of the impact of these sprays on either resistance or NTO effects.

704. In terms of how monitoring can help with mitigation of the risks as suggested by Andow and Squire, there is still a huge amount of disagreement about how best to proceed. Schemes for specific monitoring, e.g. to detect resistance, are easy to design, as are those testing specific hypotheses (such as, are there fewer parasitoids in Bt crops than conventional ones). However, significant problems arise on the question of general surveillance to detect unforeseen effects: because the effects are unanticipated it may remain unclear how they are to be detected and dealt with. EFSA has published draft guidance on general surveillance and both EFSA and the EC working group on PMM are in the process of designing improved schemes. For example EFSA have already drawn up an environmental risk assessment and monitoring plan for Bt11 maize. Other suggestions are in the ACRE guidance on PMM.

4. Resistance management

705. On the question of resistance management, apart from the submissions that it has already made, and taking into account the general comments that it has already submitted in these observations, the European Communities would like to make the following additional points. The European Communities would recall, first, that there is a significant risk, given that this is a new issue specifically relating to Bt crops (regarding continuous exposure and so on). Second, experience in the context of the use of conventional pesticides confirms how quickly problems can arise and how difficult it can be to control the situation once it has developed.

706. Data on resistance management is improving and there is less uncertainty in both the results and how to measure them. The European Communities agrees with Dr. Andow that understanding in 2003 is better than in 1997. The Spanish monitoring scheme had been in place for more than 5 years by 2003 and the EC protocol was published in 2003. There is also improved information from the US, China and Spain to show how the development of resistance may be controlled if fields are well managed and there are standard protocols for managing the risk. For example, there is the 1998 EPA resistance management protocol and the report form the EU working group on insect-tolerant crops protocol[255]. There is thus more information on baselines, monitoring procedures and length of time needed – although many of these studies are not from Europe. Baselines remain problematic in terms of general surveillance (few are available and those that are are poorly characterised and movable). The criteria for monitoring plans may be better understood, but there remain arguments about indicator species, etc. and these will vary between locations. The European Communities agrees that Dr. Andow's suggestion of a progressive determination of the risks is a sensible one, and is an idea that has been suggested in the literature many times before[256]. This tiered approach moves from lab to small scale field to larger scale field studies and there are several examples of it working very

[255] Doc Nr ENV/03/24.
[256] E.g. Poppy; Romeis.

effectively. It is also probably the only sensible way forward in terms of general surveillance and indeed it has already begun with the crops in Spain, China and the US.

E. MAIZE MON 810 (NOTIFICATION C/F/95/12-02) (AUSTRIA, ITALY) (QUESTIONS 78 TO 80)

707. The European Communities has already indicated above the fundamental criticisms it has of the approach adopted by Dr. Nutti.

708. In particular, the European Communities considers that Field data on development of Bt-resistant pests or adverse effects on NT species from cotton in USA and Australia is clearly not appropriate to determine the risks of growing Mon 810 maize in European countries, such as Austria and Italy. The crop is different (cotton v maize), the target pests are often different in different continents and countries, the NT species in EC countries are different in different countries and regions, the cultivation methods for Bt maize are different (due to climate, pest and disease pressures, scales of fields, etc), the regional environments are different (impacting on plant genotype x environment interactions), the scales of cultivation are different (size of maize fields, connectivity to non-cultivated flora and fauna), etc. Thus, such gross simplification (i.e. considering Bt cotton in USA or Australia equivalent to Bt maize in Europe or Austria) is, without doubt, seriously scientifically flawed.

709. The research by Cornell on Monarch butterflies and Bt maize pollen was considered 'inconclusive'. In these circumstances, it would be appropriate to identify knowledge gaps and uncertainties, as appropriate to the specific environment under consideration, as well as NT species and cultivation methods which warrant further scientific testing - not to simply reject the results out of hand because they are inconclusive.

710. The European Communities thus shares Dr. Andow's conclusion that scientific evidence in 1999 and 2003 was insufficient to reduce scientific uncertainties surrounding environmental impacts of Bt Mon810 if grown in the relevant Member State, to an acceptable level.

711. The European Communities would also observe that this product, like Bt 176, also contains the Cry1ab gene and many of the issues raised are the same (non-target organisms; resistance management; monitoring). The European Communities therefore here also comments on certain issues that are specific to this product. One issue which is rather different between Bt-176 and Mon 810 is the molecular characterisation. It is clear that there was uncertainty over the molecular characterisation of Mon 810 in 1998, with more information being provided by late 2003[257]. Dr. Andow raises this issue in his consideration of Bt 176, to which considerations he continuously refers in his comments on Mon 810.

712. The issues relating to resistance management and monitoring and NTOs are very similar to Bt 176. However, the post-market monitoring (PMM) plan was very poor. Even by 2003 ACRE considered that the PMM plan was not proactive enough.

713. Dr. Andow comes to the conclusion that, as in the case of BT 176, the scientific evidence available in June 1999 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to plants and the environment. Furthermore, in 2003, there remained some uncertainty about non-target risks of Mon810. Dr. Nutti answers these questions focussing mainly on resistance induction and effects on target organisms, which are environmental questions, although she indicates that this is not her field of expertise. Furthermore she only cites the statements of various Scientific Committees and agrees, but without discussing the issues.

[257] See ACRE documents and UK opinions in the relevant attachments.

714. The European Communities considers that the SCP obviously endorsed the critical arguments by asking for and encouraging fundamental new and massive instruments for safety management, such as monitoring in this area to detect any deleterious impacts. Obviously the scientific uncertainty was very high at this time. As already publicly stated, the SCP considers that it would be sensible to conduct monitoring in post-release situations. Furthermore, it strongly endorsed the practice of monitoring with appropriate and adequately targeted methodology, the large-scale introduction of such crops in order to detect any deleterious impact on non-targeted Lepidoptera and other insect populations. The SCP wished to be informed of the results of any such field monitoring studies and as relevant information continues to become available would further advise the Commission and draw its attention to any significant concerns that may arise.

715. The European Communities also notes from a US-EPA FIFRA/SAP panel that uncertainties in Bt resistance induction, models and effects on non target mechanisms are still remarkable. The European Communities also refers to the WHO/ANPA report on environmental effects of GMO where this report indicated significant concerns in the addressed areas at the relevant time.

716. In Exhibit EC 158/30, 1/2004 Austria discusses the principal problems of GM plant assessment in the fields of non target effects or allergenicity where especially the arguments of Valenta for difficulties in the assessment of allergenicity are of relevance. Austria argues (Exhibit EC 158/31) that, despite the contrary opinion of the scientific committee involved at the time, Austria's doubts for the most part continued to persist. In the case of the Bt maize varieties admitted, further doubts had in the meantime also been expressed by scientists regarding the possible allergenicity of a number of Bt proteins, in particular Bt 176 and MON 810 (and also Bt 11). In this connection, a study commissioned by Austria established that all previous applications for admission under Directive 2001/18/EC and also EC Regulation 258/1997 were inadequate in their safety assessment with respect to possible toxicological, allergenic and chronic effects of proteins formed by genetic modification. Austria therefore considered that they do not comply with the new requirements in Annex II of Directive 2001/18/EC or with the criteria of the new Food and Feed Regulation.

717. Even if a given protein *per se* does not represent an allergen, its expression in another host organism may indirectly upregulate the expression of potential allergens. It is therefore recommended to compare the engineered plant/plant product with that of the parent/wildtype plant/plant product regarding IgE reactivity to establish whether the transgenic organism represents a more potent allergen source than the parent/wildtype organism for already sensitized patients. The potentially increased ability of the transgenic organism versus the parent/wildtype organism to induce de novo IgE responses (i.e. allergic sensitization) needs to be compared by immunization experiments.

718. Concerning the results of the toxicological assessment of the companies, it must be stated that the comprehensive toxicological risk assessment as described in Spök et.al. should be carried out. The recommendations given for a standardized and harmonized approach to the generation, presentation and interpretation of data concerning toxicology and allergenicity of GM products are based on in depth scientific studies, performed by experienced scientists in the field. The proposed tests should be performed by the notifier and the resulting data provided in order to guarantee a high level of safety and public confidence in the approach taken.

719. Preliminary results of scientific research on Cry1Ab proteins and the Bt-corn/cotton leafworm/green lacewing tritrophic interaction – undertaken within the scope of the EU-project "Bt-BioNoTa" show the following unintended effects. Potential effects on non-target organisms (level of expression) were influenced by the diet chosen, i.e. artificial versus tritrophic diet. This "dietary effect" is regarded as stronger that the effect of the toxin itself. Cry1Ab increased the mortality in

green lacewing larvae both when fed directly as well as through a preherbivor (when expressed in cotton).

720. The European Communities would also note that this maize line contains an ampicillin resistance (bla gene) controlled by a bacterial promoter. Considerations detailed in the answer to general question 1 apply to this GMO. This GMO contains an antibiotic resistance gene already widely distributed in nature but confers resistance to antibiotics which are important and used for therapy in defined areas of medicine. Since it is rather difficult to have a quantitative estimation of the risk for human health and since there are still controversy on the risk, it seems preferable to use this GMO only for limited trials in field but not for commercialization.

721. There is a similar environmental case as for Maize Bt 176. This consent is for cultivation, so comments on the environmental impact of Bt on target, and non-target organisms and the development of resistance in corn borers have much phytosanitary relevance, since they will be exposed by the growing of Bt maize in Austria or Italy.

722. Austria expressed concern that these issues of the environmental impact of Bt on target, and non-target organisms and the development of resistance in corn borers had not been properly addressed by various committees across Europe. Scientific papers were appearing which appeared to show some adverse impacts on non-targets. Resistance development was considered an agricultural problem by these committees where as Austria saw resistance development as an environmental issue (and under current EU regulations it is now considered as an environmental issue). Strategies for managing resistance in corn borer were only beginning to be developed in Europe.

723. Dr. Andow observes that, with reference to the SPS Agreement, Austria has evaluated relevant risks to plant health. Specifically, Austria has taken into account available scientific evidence. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument, and that argument holds for this case because Austria argues that there is a plant pest risk. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. Furthermore, the reasoning of Austria is consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

724. In conclusion, Austria and Itlay had several phytosanitary grounds for requesting a ban on cultivation of maize Mon810, which are mostly supported by Andow:

the environmental impact of Bt non-target organisms;

the development of resistance to Bt in corn borers; and

the lack of clear strategies for managing resistance in corn borer.

725. Austria and Italy felt that these issues had not been properly addressed by various committees across Europe while scientific papers were appearing which appeared to show some adverse impacts on non-targets and Resistance development was considered an agricultural problem by these committees and guidelines were only beginning to be developed.

726. Dr. Nutti states that the issues at stake concerned undesired effects on NT organisms and development of resistance in insects based on studies of other Bt plants in other continents. Clearly she admits that she is not an expert in this area and her answer is inadequate to address the scientific concerns raised.

727. More recent studies on possible environmental impacts of Bt crops[258] may provide the basis (risk assessment tools) for appropriate risk management studies in future, at least in a generic sense. However, these would need to be modified and validated for each Member State so that they were appropriate (i.e. used to identify most regionally important NT species or ecological functions, most regionally appropriate cultivation and crop management systems, most appropriate crop and management systems as 'baselines' to compare levels of risk and benefit).

728. The Codex Guidelines for GM foods to include analysis of unintended effects. "The broadening of the Codex risk assessment to include indirect effects (e.g. unintended changes in GM crops) now encompass effects of novel foods on the environment that have an indirect impact on human health". Human health is viewed as an 'integrating index of ecological and social sustainability', as outlined by the WHO and the National Agency for Protection of the Environment in Rome (2000) when reviewing potential environmental hazards of GM crops. These findings were based on peer reviewed studies of GM barley, canola, maize, oilseed rape, potato and rice, published between 1994-2001[259].

729. Hence the European Communities disagrees with Dr. Nutti's analysis. There were several scientific uncertainties in 1998 regarding environmental harm and animal nutrition (interpreting animals in its broadest sense to include a range of NT organisms). Levels of protein in GM crop events are only one of several indicators of unintended changes during transformation which could affect Italian NT organisms (others would include sugars, amino acids and plant secondary metabolites known to be ecologically important to most insect species).

730. These uncertainties were in part confirmed later in 2003[260], when several unintended effects in GM crops were reported which would require testing under Italian conditions (i.e. regionally relevant NT species, crop management systems, environments etc – see my earlier responses for references).

731. Dr. Nutti often concludes on environmental issues and refers to CODEX/ FAO/WHO guidelines, materials. However, Codex Guidelines do not relate to the environmental issues. The European Communities refers to a published paper[261] which addresses the point that Codex Guidelines might include the assessment of unintended effects in the environment as a prerequisite for health assessment. This could include e.g. the need of an assessment of effects in the environment (such as gene flow) which is relevant for assessing GM health and food safety in case contamination from products without foods safety assessment enter the food chain.

732. According to Haslberger, the development of risk assessment concepts reflected the progress in the understanding of possible unintended effects of biotechnological methods in breeding. Early regulations (e.g. EC, directive 90/220) for GMO did not differentiate between environmental and product specific risks assessments whereas most modern regulations differentiate between a general environmental assessment and assessments for specific products, such as pharmaceuticals, foods and feeds, seeds, chemicals or even fibre products[262]. Specific risk assessment procedures were developed

[258] Eg. from the GMO Guidelines Project; Birch et al., 2004 In: Environmental Risk Assessment of Genetically Modified Organisms vol 1. a case study of Bt maize in Kenya. Eds Hilbeck and Andow. CABI Publishing. pp 117-1850, and further case studies in press.

[259] See Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741 for summary.

[260] See Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741.

[261] Ibidem.

[262] EC, Biotechnology, 2004.

for these products. This specification resulted into a diversification for the assessment; however, experiences drawn from a growing body of risk assessment processes often indicate similar underlying problems. Notably the need for a molecular characterisation and assessment of potential unintended molecular effects was identified as the basis for the assessment in all fields.

733. The concept that a comparison of a final product with one having an acceptable standard of safety provides an important element of safety assessments of GMOs was a commonly used basis for the development of both, food safety and the environmental risk assessment[263]. This concept was elaborated by FAO, WHO and OECD in the early 1990s and referred to as substantial equivalence for the assessment of GM foods. But in 2000 an FAO/WHO consultation acknowledged that this concept had attracted criticism from the perception that it was the end-point of a safety assessment rather than the starting point[264]. The consultation concluded that a consideration of compositional changes is not the sole basis for determination of safety and that safety can only be determined when the results of all aspects under comparison, and not merely comparisons of key constituents are integrated. More recently the concept has evolved to a Comparative Safety Assessment for GMO foods[265]. By 2003, both international systems covering GMO, GM food safety (Codex) and environmental safety (CPB) became effective, where both systems are based on the concept of a case by case approach. More recently, the need for a comprehensive molecular characterisation of each transformation event, including the analysis of integrated constructs and the flanking region as well as the need to address potential unintended effects was appreciated for food safety- and environmental assessments[266] and this idea was also enforced by general recommendations of a US-FIFRA expert panel[267].

734. The Codex Alimentarius Commission adopted the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, and the Draft Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants and Microorganisms (Codex). The principles for the safety assessment dictate a case by case pre-market assessment on the basis of a comparative safety assessment (CSA). The CSA is basically a two-tiered approach. The initial step is comprised of a thorough comparison with the closely related conventional food organism counterpart to identify any differences that may have safety implications for the consumer. This comparison includes both phenotypic characteristics as well as a compositional analysis. The second step comprises the toxicological and nutritional evaluation of the identified differences between the food derived from the GMO and its comparator. Hazard identification and characterization are typically the first steps in any risk assessment and an extensive molecular characterisation of the inserted

[263] WHO, 1991. Strategies for Assessing the Safety of Foods Produced by Biotechnology, Report of A Joint FAO/WHO Consultation. World Health Organisation, Geneva. Available at <http://www.who.int/foodsafety/publications/biotech/1990/en/>.

[264] FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, 29 May - 2 June 2000. Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/es/esn/food/gmreport.pdf; Millstone, E., Brunner, E., Mayer, S., 1999. Beyond 'substantial equivalence'. Nature 401, 525-526; Schenkelaars, P., 2002. Rethinking substantial equivalence. Nature Biotechnology 20, 2, 119. Canadian Royal Society, 2002

[265] Kok, E.J., Kuiper, H.A., 2003. Comparative safety assessment for biotech crops. Trends in Biotechnology 21, 439-444.

[266] FAO/WHO, Safety assessment of foods derived from genetically modified animals, including fish, a joint FAO/WHO expert consultation on food derived from biotechnology, Rome, Italy, 17 - 21 November 2003. Available at <http://www.who.int/foodsafety/biotech/meetings/en/gmanimal_reportnov03_en.pdf>.

[267] SAP Report No. 2004-05. MINUTES of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) Meeting, June 8-10, 2004, Arlington, Virginia. A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management For Bacillus thuringiensis (Bt) Cotton Products. 84 pages.

genetic material construct is required. The safety of the gene product must be assessed on a case-by-case basis. Following the phase of hazard identification, characterization and food intake-assessment an integrated toxicological evaluation will combine all the information with relation to the food safety of the GMO-derived food. For the identification of potentially occurring unintended effects, profiling methods have been proposed and different possibilities for profiling methods have been characterized[268]. In addition to investigating health risks directly associated with food production, the broadening of the Codex risk assessment to include indirect effects now encompass effects of novel food on the environment that may have an indirect impact on human health[269].

735. A case by case assessment considering any organisms derived from a transformation event as well as different receiving environments is broadly recognised as the best framework for assessing environmental risks of GMOs. Internationally the concept of familiarity was developed also in the concept of environmental safety of transgenic plants. The concept facilitates risk/safety assessments, because to be familiar, means having enough information to be able to make a judgement of safety or risk[270]. Familiarity can also be used to indicate appropriate management practices including the evaluation whether standard agricultural practices are adequate or other management practices are needed to manage the risk[271]. As familiarity depends also on the knowledge about the environment and its interaction with introduced organisms, the risk/safety assessment in one region may not be applicable in another.

736. Currently The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity is the only international regulatory instrument which deals specifically with the potential adverse effects of GMOs on "the conservation and the sustainable use of biological diversity", an important amenity of the environment, taking also into account effects on human health. The CPB covers transboundary movements of any genetically modified foods that meet its official definition of living modified organisms (LMOs). Article 11 of the CPB asks for a risk assessment where annex III of the Protocol specifies general principles and steps for environmental risk assessment of LMOs and focuses especially on identification of any novel genotypic and phenotypic characteristics that may have adverse effects on biological diversity in the likely potential receiving environment. Annex III recommends that the risk assessment should also take into account risks to human health and evaluate the likelihood of these adverse effects. The information on the receiving environment includes data on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment. As the focus of the CPB is biodiversity, in line with the scope of the Convention itself, its consideration of human health safety is limited, concentrating on situations in which an LMO itself may end up in the food supply, such as might happen via trade of crop seeds[272]. Pharmaceuticals are explicitly excluded.

737. Recent work for the implementation of the CPB recommends the analysis of effects of GMOs on species in the environment before an assessment of effects on the biodiversity for the assessment of non target environmental risks. This mainly because of a better access to species assessment and

[268] Kuiper et al., 2003.

[269] Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741..

[270] Environmental Effects of Transgenic Plants. The Scope and Adequacy of Regulation. National Research Council, 2002. National Academy Press, Washington, 320 pp.Conner et al., 2003

[271] OECD, 1993.

[272] Miraglia, M., Berdal, K.G., Brera, C., Corbisier, P., Holst-Jensen, A., Kok, E., Marvin, H.J.P., Schimmel, H., Rentsch, J., van Rie, J.P. & Zagon, J. (2004; alphabetical order). Detection and traceability of genetically modified organisms in the food production chain. Food Chem. Toxicol. 42: 1157-1180.

methodological limitations of an analysis of effects to diversity[273]. Specifically a non target risk assessment for non target effects of GM crops was discussed to be more useful than the conventionally used eco-toxicology or non-indigenous-species model. This new model requires information about the intended receiving environment and aims to focus on the analysis of possible effects of local species with a specific ecological function in this system.

738. Both, the Codex principles for food safety and the risk assessment provisions of the CPB provide opportunities to more explicitly consider interactions between food safety and environmental safety. The broadening of the Codex Risk assessment to include indirect effects provides for assessing effects on the environment that may have an indirect impact on human health[274].

739. In the explanatory guide to the CPB (CPD, explanatory guide) indirect effects on the environment are described as effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management. Observations of indirect effects are likely to be delayed. Given examples include impacts which can arise from changed agricultural practices associated with the management of a genetically modified (GM) crop rather than from the genetically modified crop itself. The explanatory guide suggests that questions related to human health effects were intended to be covered by the Protocol only if the human health aspect is linked to biological diversity. Such a link exists if the health effect is consequent to exposure *in situ*, for instance, if a farmer were to develop an allergenic reaction to pollen from genetically modified plants; it also exists if the health effect resulted from effects on biological diversity (secondary effect). Direct effects on human health (e.g. caused by consumption of GM food) would, however, not be covered by the Protocol.

F. MAIZE T25 (NOTIFICATION C/F/95/12-07) (AUSTRIA, ITALY) (QUESTIONS 84 TO 89)

740. The European Communities has already outlined above its fundamental criticisms of the approach adopted by Dr. Nutti.

741. The European Communities considers each GM crop event should be examined on a case by case basis, as stated in the Cartegena Protocol. This means that Member States are justified in requesting region specific testing, since each GM event can respond differently to regional differences in environment (weather, soil types etc), regional pest and disease pressures, regional agronomic practices (e.g. pesticide and herbicide use, tillage systems), regional abundance of flora (including weeds which could become resistant to herbicides) and fauna (which could be affected by any unintended changes in the GM crop).

742. The European Communities agrees with Dr. Andow that secondary ecological effects (longer term over several seasons and indirect effects resulting from the GM crop and its management system) need to be assessed for each Member State under regionally appropriate conditions.

743. For GMHT crops, the UK Farm Scale Evaluation (FSE) trials indicated particular risks of using HT crops in combination with particular herbicides and particular crop management systems. For maize no adverse effects were detected for GMHT maize, but the comparison was with conventional maize used with the herbicide atrazine. This herbicide has known adverse effects on the environment and NT organisms and will be banned in the UK shortly, so was widely criticised as a

[273] Andow & Hilbeck (2004). Science-based risk assessment for non-target effects of transgenic crops. BioScience 54: 637-649. Birch et al., 2004 In: Environmental Risk Assessment of Genetically Modified Organisms vol 1. a case study of Bt maize in Kenya. Eds Hilbeck and Andow. CABI Publishing. pp 117-1850..

[274] Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741.

poor/inappropriate choice as a 'control treatment' for comparison with GM HT maize in the UK FSE trials.

744. Hence the European Communities considers that regionally appropriate combinations of the GMHT crop, herbicides, pesticides and agronomic practices need to be tested in comparison with an appropriate baseline combination of these factors (e.g. to detect beneficial or adverse changes in regional biodiversity on herbivorous species feeding on weeds affected by the GMHT crop management system).

745. Co-existence (and related contamination levels in seeds) is a relatively new issue, although even here scientific uncertainties about distances of GM gene flow and establishment for several GM crops including maize were widely discussed and strongly debated Europe for several years prior to 2003.

746. The European Communities would agree with Dr. Andow that in 2003 maize T25 was inadequately characterised regarding Austria's needs on risk management in relation to possible effects on regionally import NT species, on regional gene flow, on regionally important weed control issues and agronomic practices and on possible contamination issues if GM genes are introgressed into conventional or organic maize in Austria, or other Member States. Johnson[275] provides a set of generic questions which EC Member States could now adapt to carry out a more scientifically rigorous risk assessment of GMHT crops and a review of regionally-appropriate management strategies.

747. The European Communities observes that the analysis of Dr. Andow is differentiated and science based. He argues that the amended SCP Opinion (CDA-77, 20 July 2001) does not provide an assessment of environmental risks beyond gene flow risks. These would include non-target and other biodiversity risks. The amended SCP Opinion (CDA-77, 20 July 2001) does not provide an assessment of this environmental risk. Even though the Member States did not provide any scientific evidence that this risk exists for T25 maize, resistance risks are widely recognized, and the consistent use of glufosinate with T25 maize would result in a resistance risk. Thus, there was insufficient scientific evidence available to the SCP and Austria and Italy to assess weed resistance risk and appropriate risk management measures.

748. The Member States expressed the view that risks from gene flow (dispersal) is small but still must be monitored. This is especially true for specific regions. Here a concept for specific regions, sensitive areas, is specified, scientifically cited and explains why a monitoring is especially necessary in the light of risks to specific areas/regions. As no monitoring concept was available Austria's concern was justified.

749. Furthermore, special measures monitoring the possible – mostly regarded as safe – spread of pollen to fields in the surroundings cultivated with conventional maize are missing.

750. The lack of a monitoring programme regarding the long term effects of genetically modified plants and herbicides can be criticised especially because of the fact that the approval conditions do not foresee a protection of sensitive areas. Furthermore, regional ecological aspects are not differentiated: the use of herbicide resistant plants in areas of unavoidable applications of herbicide seems to be useful, if good agricultural practice minimizes the danger of a resistance development.

[275] Johnson, Blancas and Borem (2004) (CABI book: 'Environmental Risk Assessment of GMOs: A case study of Bt maize in Kenya'.

751. This concerns of Austria and Italy were even more substantiated when science in the following years elaborated hazards from gene flow and dispersal, also in maize, addressing especially problems in the area of co-existence. Co-existence arguments were addressed by Austria in detail in subsequent discussion. Some points of the opinion of the SCP presented in 2000 may need to be reflected by subsequent scientific findings on gene flow.

752. At the time of this application, concerns about the ecological impacts of the herbicides used on HT crops was high and UK had implemented the Farm Scale Evaluation programme[276] to examine these effects in maize and other crops.

753. There are at least 6 reasons for the safeguard measures. The reasons are: (1) The environmental risks of T25 have not been sufficiently evaluated under realistic conditions in Europe. (2) There is no post-commercialization monitoring program. (3) Although harm from pollen transfer is likely absent, the potential for gene flow to conventional maize production fields needs to be quantified and included in monitoring because of its agricultural consequences. (4) There is no provision for the protection of ecologically sensitive regions. (5) There is a need for "good farming practice" guidelines to minimize the danger of herbicide resistance. (6) There is a need to assess long-term and secondary ecological effects, especially those due to changes in herbicide management.

754. With reference to the SPS Agreement, the Member States have evaluated a risk relevant to plant health. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument, and that argument holds for this case because Austria argues that there is a plant pest risk. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. The reasoning of Austria and Italy is consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

G. MAIZE MON 809 (NOTIFICATION C/F/95/12-01/B) (ITALY) (QUESTIONS 90 TO 92)

755. The reasons why Italy declines to authorise the placing on the market of this product refer especially to the risk assessment based on substantial equivalence (see the previous discussion of that question in these comments), to international discussions and criticisms of this principle also under CODEX, and to changes of the use of the principle and the legislation:

> "… Having regard to the subsequent opinion of the Superior Institute of Health, dated 28 July 2000, addressed to the Minister of Health, which addresses the concept of "substantial equivalence" from a technical-scientific viewpoint and states that, if interpreted from a legal viewpoint, the concept could be defined differently, given the lack of clarity in the Community legislation;
>
> Considering that the Superior Institute of Health, in its opinion, pointed out that, of the seven notified products cited above, the four types of maize contain levels of protein deriving from the genetic modifications ranging from 0.04 to 30 parts per million and that, therefore, the foodstuff has been permanently affected by the modified elements;
>
> Considering also that, in the same opinion, it is reported that the technical documentation available does not examine the GMOs in comparison to their conventional counterparts with regard to the presence of certain micro-constituents,

[276] Firbank et al 2003 e.g. Rieger et al., 2002. SQUIRE, G.S., BROOKS, D.R., BOHAN, D.A., CHAMPION, G.T. et al. (2003) On the rationale and interpretation of the Farm Scale Evaluation of genetically modified herbicide-tolerant crops. Philosophical Transactions of the Royal Society B, 358, 1779-1800..

> and it finds a substantial identification with the conventional counterparts from a purely nutritional standpoint (micro and macronutrients) but not from the standpoint of the composition of the product, as foreseen in the Community regulations, because of the presence of modified molecules;
>
> Whereas, in the aforementioned opinion, the Superior Institute of Health also comes to the conclusion that, in the light of current scientific knowledge, there are no apparent risks to the health of humans or livestock from the consumption of derivatives of the aforementioned GMOs, but that this conclusion is reached in a context in which, from the correspondence with the President of the European Commission and with the pertinent European Commissioner, it is evident that there are inadequacies in the risk assessment procedures;
>
> Whereas, furthermore, in the aforementioned opinion, the Superior Institute of Health declines to express an opinion regarding the risk of possible "environmental release" of the GMOs in question, even when it has been ascertained that residues of modified components remain in the foodstuffs, so that the inadequacy of information deriving from the previous phase of preliminary assessment of this environmental release for the simplified authorization procedure appears to be even more deleterious to the precaution principle, generally adhered to in these matters;"

756. The European Communities notes that Dr. Nutti agrees with the opinion of the Italian CA regarding the ambiguity of the term "substantial equivalence", and agrees that this concept could be defined differently. In this respect, the European Communities notes that the assessment of the Italian arguments by the SCF (in CDA 86) did not directly address in detail the substantial equivalence issue.

757. The European Communities also notes that there are some important toxicological issues that have not been addressed by Dr. Nutti. For instance, the recently published observation on occupational allergy to Bt bacterium spores in farmers using Bt pesticides mentioned by the SCF and by Italy. Furthermore, the Italian argument concerning the risk of possible environmental release of the GMOs in question, even when it has been ascertained that residues of modified components remain in the foodstuffs is not discussed by Dr. Nutti. Thus, in essence substantial disagreement remains on the question of substantial equivalence. It would appear that Italy effectively anticipated a change in the relevant scientific and legal elements.

758. The concept of substantial equivalence is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant-DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its conventional counterpart.

759. The misleading conclusion that a risk assessment according to substantial equivalence *per se* can establish safety was corrected explicitly in 2000 when an FAO/WHO consultation acknowledged that this concept had attracted criticism from the perception that it was the end-point of a safety

assessment rather than the starting point[277]. The Consultation concluded that a consideration of compositional changes is not the sole basis for determination of safety and that safety can only be determined when the results of all aspects under comparison, and not merely comparisons of key constituents, are integrated. More recently the concept has evolved to a Comparative Safety Assessment for GMO foods[278].

760. Thus, in the light of its chosen level of protection, the European Communities considers that, as a matter of WTO law, Italy was justified, before carrying out analysis on substantial equivalence, to require access to data describing the molecular make-up of the transgenic events. This is necessary in order to have a good description of the plant material used in the comparative analysis (e.g. chemical composition, nutritional value, etc.).

H. MAIZE BT-11 (REFERENCE C/GB/96/M4/1) (ITALY) (QUESTIONS 93 TO 95)

761. The European Communities refers to its observations with regard to the preceding product, as well as its general observations and its observations in the context of the alleged product specific delays, which apply *mutatis mutandis*. The European Communities has the following additional remarks and conclusions.

762. The information requested and supplied from the company was mixed, scarce, delivered consecutively all over years, and not convincing. The quality of the dossier can therefore be considered as not sufficiently informative, taking into consideration the specific legislator's chosen level of protection. The major weaknesses of the dossier relate to:

No sufficient experimental evidence to assess the safety;

Compositional data insufficient for a product directly consumed by human;

No *in vivo* experiments conducted on laboratory or farm target animals with grain of the event "sweet maize";

Field maize used as a control - grain material was spiked with Bt proteins (resulting in poor and unsatisfactory experimental conditions);

Further experiments performed on ruminants using the whole plant silage or stalk have no meaning for the safety assessment.

763. Taking into account the legislator's chosen level of protection, these issues could be considered to have justified requests for further evidence on the safety of the product.

[277] FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, 29 May - 2 June 2000. Food and Agriculture Organisation of the United Nations, Rome. ftp://ftp.fao.org/es/esn/food/gmreport.pdf; Millstone, E., Brunner, E., Mayer, S., 1999. Beyond 'substantial equivalence'. Nature 401, 525-526; Schenkelaars, P., 2002. Rethinking substantial equivalence. Nature Biotechnology 20, 2, 119. Canadian Royal Society, 2002

[278] Kok, E.J., Kuiper, H.A., 2003. Comparative safety assessment for biotech crops. Trends in Biotechnology 21, 439-444.

VII. THE PANEL'S QUESTIONS ON ISSUE 3 ("LIKENESS") (NOS 96 TO 109)

Question 96

Has there been a significant change in the understanding of physical, chemical and biological characteristics of biotech products, gene interactions, gene expression, gene silencing, molecular characterization, and product specific detection since 1998?

General comments

764. The Panel has received responses from Dr. Nutti and Dr. Squire, indicating a difference of approach. Dr. Nutti makes clear that she excludes from her response any view in relation to "any environmental issue", which she states is "not my area of expertise". On the matters within her expertise she states that "the approach remains essentially the same". The European Communities considers that this does not provide a clear or unambiguous response to the question posed.

765. Dr. Squire considers that "most of the underlying molecular and biochemical knowledge of the subject has not changed substantially during this period." However, he makes it clear that the absence of substantial change should not be taken as an inherently positive development in the state of scientific understanding. As he puts it: "There is still uncertainty over unintended side-effects occurring in biotech products, and the continued stability of phenotype in biotech plants or continued expression of their genes outwith the crop itself (i.e. in volunteers and feral plants)." The Communities understands this to mean that the absence of substantial change indicates continued levels of uncertainty.

766. The European Communities considers that there has been a significant change in the understanding of the issues addressed by the question.

Detailed comments

767. Although, the basic principles of risk assessment remain unaltered, there has been a significant change in the understanding of most of the characteristics listed in the question. This is primarily because of the accumulation of considerably more data and the development of more effective analytical tools for characterization, data mining and data comparison.

768. The Communities would refer by way of example in the food safety domain to a research project - "ENTRANSFOOD, European network - safety assessment of genetically modified food crops" – which was launched in 2000 to identify key issues of the safety evaluation of genetically modified food crops, and to examine whether current research methods were adequate to characterize specific safety hazards. The project was concluded in 2003, and a series of position papers were published in a special volume of Food and Chemical Toxicology.[279]

769. The first position paper[280] provides an updated approach for adapting the test strategy to the characteristics of the modified crop and the introduced trait, and assessing potential unintended effects from the genetic modification. The second position paper[281] focuses more specifically on detection and characterization of unintended effects in GM crops. It pays particular attention to new profiling

[279] G. van den Eede, H. Aarts, H.-J. Buhk, G. Corthier, H.J. Flint, W. Hammes, B. Jacobsen, T. Midtvedt, J. van der Vossen, A. von Wright, W. Wackemagel, A. Wilcks. Food and Chemical Toxicology 42 (2004), 1127-1156. (Volume 42, issue 7, July 2004).

[280] König et al., 2004.

[281] Cellini et al., 2004

technologies, including metabolomics, proteomics and transcriptomics. The background for the project was that significant developments had been made with respect to the scientific understanding of underlying biological, physical and chemical mechanisms in general, and particularly biological, physical and chemical characteristics of biological products, including biotech products. Considerably more is known since 1998 about gene interactions and gene expression. The approaches applied to molecular characterization are different, in particular they are much more efficient and sensitive, and allow us more efficient data mining. Information made available in e.g. genome and protein sequence databases has made possible assignment of function to numerous sequences characterized in e.g. plant species by comparison with characterized sequences of related plant species. One example is provided in this report under EC chronology 63, exhibit 63/119.

770. As is clear elsewhere, there has also been advances of several orders of magnitude in the development and availability of product specific detection (see comments on question 8), methodologies for genetic modification characterization (see comments on question 9), and identification of unintended effects thereof (see comments on question 111).

Question 97

On the basis of the information before the Panel, is there new scientific evidence since 1998 that would suggest that the potential risks to human, plant or animal health, or to the environment, from any of the specific biotech products subject to this dispute (including products subject to the member State safeguard measures), are different in nature or magnitude as compared to the scientific understanding of the risks associated with such biotech products prior to 1998, taking into account:

- *the intended use of each product (direct human or animal consumption, further processing for consumption, planting or any other specified use);*

- *any potential risks that may arise from the combination or successive use of biotech products.*

Does the information before the Panel support the view that the potential risks from the products in this dispute should be assessed differently than the risks from biotech products approved prior to 1998?

General comments

771. The European Communities notes that there is support amongst the independent experts for the view that the potential risks to human, plant or animal health, or to the environment, from some of the specific biotech products subject to this dispute (including products subject to the member State safeguard measures), are different in nature or magnitude as compared to the scientific understanding of the risks associated with such biotech products prior to 1998. The information before the Panel supports the view that potential risks from some of the products in this dispute should be assessed differently than the risks from biotech products approved prior to 1998.

Detailed comments

772. The Panel has received responses from Dr. Snow, Dr. Andow and Dr. Squire which are broadly consistent, and a response from Dr. Nutti which adopts a different approach. Dr. Nutti once again excludes any consideration of environmental risks from her response. In relation to the remaining matters she concludes that:

> "My opinion is that, based on the existing literature and knowledge on the subject, and from a scientific perspective, there is **no** new evidence that would suggest that the potential risks to human, animal or plant health, from any of the specific biotech products subject to this dispute, are different in nature or magnitude as compared to those products of biotechnology approved by the European Communities prior to October 1998."

773. In relation to environmental risks Dr. Snow reaches a different conclusion:

> Yes, new scientific evidence has become available since 1998 with regard to gene flow from herbicide-tolerant crops and taking into account the environmental concerns and management goals of EC Member States. New scientific studies published during 1998-2003 showed that the dispersal of transgenes that confer resistance to glufosinate and glyphosate will occur much more widely and more quickly than was previously expected, as I discuss in Part I. Also, new studies showed that the widespread use of glufosinate-tolerant and glyphosate-tolerant oilseed rape could lead to populations of volunteers and weeds that are more difficult to manage than their nontransgenic predecessors. Adopting these herbicide-tolerant crops could lead to greater dependence on these and other herbicides (see Part I and Friesen et al. 2003). These problems would not arise without the use of transgenic crops because the genes that confer resistance to glufosinate and glyphosate have not been found to occur naturally in the crop's gene pool." She concludes her response as follows: "In summary, the types of new scientific information that I discuss in Part I of my answers can be used to support the view that potential risks from the products in this dispute could be assessed differently than the risks from biotech products approved prior to 1998.

Dr. Squire concludes that

> knowledge of the ecological impacts of biotech products and their spread and concentration in the agricultural environment has changed, particularly in that emergent properties at the scales of the field and landscape are now much better appreciated even if they are still far from fully understood.

774. He provides several examples to support that conclusion.

775. Dr. Andow provides a detailed response which concludes that "risk assessment methodologies and evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1998 to 2005." These changes have come about "from increased scientific knowledge about transgenic crops. This has affected risk assessment for transgenic crops intended for planting in the environment. Scientific investigation of risks of combination biotech products has lagged behind." According to Dr. Andow the basis for risk assessment of transgenic crops has changed

> because several significant scientific points had come to light. (1) It became widely appreciated that the molecular basis of transformation was more complex than originally thought, and the implications of these findings for risk assessment were articulated. (2) Non-target risk assessment shifted from assessing indicators of environmental risk to assessing actual identified potential environmental risks. Presently this is done on an ad hoc basis, as no systematic methodology has gained widespread acceptance. (3) Gene flow risk assessment has shifted from being based

> primarily on an assessment of the probability of gene flow to being based on an assessment of both the probability of gene flow and the conditional hazard probability. (4) Resistance risk is considered an environmental risk, and science-based resistance management measures are required.

776. The European Communities notes that the responses of Dr. Snow, Dr. Squire and Dr. Andow are consistent with the concerns of the European Communities in relation to environmental risks, and that they justify the case-by-case approach to which the Communities is committed. The consensus amongst them is that the whole field is very complex, that we do not fully understand it, that some of the hazards predicted pre-1998 are beginning to emerge. These effects include the molecular basis of the transformation, horizontal gene flow, resistance and problems with non-target species.

777. With regards to non-environmental risks, the Communities does not share the views of Dr. Nutti. In particular, on the basis of existing research (and the absence of any exposure data on which Dr. Nutti or other might rely) it is impossible to know whether the introduction of GM food had had any human health effects other than acute toxic reactions. Therefore it is impossible to comment on any changes that might have occurred since 1998.

778. As regards research which has been carried out, this indicates a growing understanding of the inadequacies of risk assessments which have been carried out on GM foods, having regard to the uncertainties already referred to: see Freese and Schubert.[282] The scientific concerns noted by Freese and Schubert (and which have been shared by many other scientists for several years) relate to:

- the use of surrogate Bt toxins to test for allergic, toxic or environmental NT effects;
- unintended effects being ignored or deemed unimportant by US assessors[283] when there are several peer reviewed publications on such effects in GM crops;
- test protocols for risk assessment of potential human health aspects of GM crops are not considered rigorous by these authors;
- comparisons with Bt sprays are considered invalid because of major differences with Bt crops in terms of Bt toxin types, exposure routes, exposure times, thermostability, digestive stability, etc;
- metabolic profiling methods deployed to date are largely only semi-quantitative and often fail to accurately measure smaller, biologically active secondary metabolites in GM plants (which can change as a result of transformation);
- metabolic stress responses of GM crops grown in particular environments have been largely ignored by US assessors;
- chronic (long term) tests on GM crops and GM products are often lacking or poorly executed;

[282] Review published in Nov 2004, covering literature from 1991-2003 – Biotechnology and Genetic Engineering Reviews 21, 299-324

[283] See also Haslberger, A.G., 2003. Codex guidelines for GM foods include the analysis of unintended effects. Nature Biotechnology 21, 7, 739-741.

- the effects of different genetic backgrounds and growing conditions (determining plant genotype x environment interactions) for GM crops has been largely ignored, although many scientists have expressed concerns about lack of data in this area; and

- the effects on human health post-release requires properly conducted epidemiological studies, comparing GM food intake as a treatment with intake of conventional food not contaminated with GM products, or with organic food not contaminated with GM products. There do not appear to be any such epidemiological studies on human populations having been published in peer reviewed journals.

Question 98

From a scientific perspective, is there a significant difference in risks to human, animal or plant health or the environment arising from the use of a bacterial antibiotic resistance marker gene, or part thereof, in any biotech product at issue in this dispute (e.g., Monsanto Bt cotton (531), Monsanto Roundup Ready cotton (RRC1445), Amylogene starch potato) compared to those products of biotechnology approved by the European Communities prior to October 1998?

General comments

779. The European Communities notes that there is support amongst the independent experts for the view that there may be significant difference in risks to human, animal or plant health or the environment arising from the use of a bacterial antibiotic resistance marker gene, or part thereof, in some of the biotech products at issue in this dispute as compared to those products of biotechnology approved by the European Communities prior to October 1998, having regard to changes in the state of scientific understanding.

Detailed comments

780. The Panel has received a response from Dr. Nutti, which does not address environmental risks. She states that "There have been numerous experiments aimed at evaluating the possibility of transfer of plant DNA to microbes and mammalian cells. To date, there are no reports that marker genes in plant DNA transfer to these cells." As noted and commented under question 1, this statement which is taken from her reply to question 1, is absolutely not correct. She notes, however, that "Even so, the use of alternative transformation methods, which do not use ARMG, is encouraged. If alternative marker genes are used, they also must be evaluated regarding their safety." And she then concludes:

> My opinion is that, based on the existing literature and knowledge on the subject, and from a scientific perspective, there is no new evidence that would suggest that the potential risks to human, animal or plant health arising from the use of a bacterial antibiotic resistance marker gene, or part thereof, from any biotech product at issue in this dispute (e.g., Monsanto Bt cotton - 531, Monsanto Roundup Ready cotton - RRC1445, Amylogene starch potato) are different in nature or magnitude as compared to those products of biotechnology approved by the European Communities prior to October 1998.

781. The European Communities notes that Dr. Nutti accepts that the use of marker genes other than ARMG "is encouraged". The Communities considers that the evidence indicates that the use of a bacterial antibiotic resistance marker gene, or part thereof, may contribute to a significant difference

in risk including by means of transfer,[284] and that precautionary measures are therefore justified. The Communities notes that evidence of the risk of transfer only began to emerge in the scientific literature in 1998 and in following years, and that no studies were available prior to 1998. A lot of the relevant research is referred to in the ENTRANSFOOD publication.[285] The European Communities refers to its comments on questions 1 and 2 above.

782. The presence of antibiotic resistance genes in some of the biotech products at issue in the dispute (Monsanto Bt Cotton, Monsanto Round Ready Cotton, Amylogene starch potato, Maize T25, Maize MON 810, Maize Bt-176, Topas 19/2, clearly makes a difference as compared to older products, and this difference has been examined for its medical impact. The evidence is still controversial.

783. For risk analysis, the risk should not be taken in general but analysed on a case-by-case basis. Two parameters are to be considered: the existing spread of the antibiotic resistance marker in bacteria isolated in man, in animals and in the environment, and the relevance of the concerned antibiotic(s) for human therapy. Based on these parameters, the lowest degree of risk (although not zero) is represented by the *npt*II gene (kanamycin resistance), and a higher degree of risk by the *bla* and the *aadA* genes. The qualification of "higher" risk for the later category is justified by the fact that these genes confer resistance to important antibiotics. These genes have already spread, but so far, no experiment has provided data to quantify the contribution of these resistance genes to the increase of resistance genes in pathogenic bacteria. The assertion that the transfer is null or very low is not documented and this question remains open. The *bla* Topas 19/2 and Amylogen Starch potato contain the *npt*II gene and Maize MON 810, Maize T25, Maize Bt-176 contain the *bla* gene (ampicillin resistance).

784. The Communities considers, in accordance with the relevant Codex guidelines, – and Dr. Nutti appear to agree, albeit less strongly – that there is no justification to maintain these unnecessary resistance genes where alternatives exist, and that ARMG coding for resistance to antibiotics of clinical use, should not be present in GM products.

785. Finally, the European Communities wold like to indicate, that new ARMG were to be present in GM product assessed between 98 and now, some of them being though to present significant human health threads, such as nptIII, encoding for amykacin resistance, an antibiotic of critical importance in certain pathological conditions.

Question 99

For those biotech products at issue in this dispute for which no significantly different nature or level of risk has been identified, does the information before the Panel provide a scientific or technical rationale for monitoring the occurrence of potential adverse effects, or of unintentional effects,

[284] See: de Vries J, Wackernagel W. Integration of foreign DNA during natural transformation of Acinetobacter sp. by homology-facilitated illegitimate recombination. Proc Natl Acad Sci U S A. 2002, 99:2094-9; Gebhard F, Smalla K. Transformation of Acinetobacter sp. strain BD413 by transgenic sugar beet DNA. Appl Environ Microbiol. 1998, 64:1550-4 ; Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother. 2004,38:1449-59; Nielsen KM, Bones AM, Smalla K, van Elsas JD. Horizontal gene transfer from transgenic plants to terrestrial bacteria--a rare event? FEMS Microbiol Rev. 1998, 22:79-103; Nielsen KM, van Elsas JD, Smalla K. Transformation of Acinetobacter sp. strain BD413(pFG4DeltanptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. Appl Environ Microbiol. 2000, 66:1237-42.

[285] G. van den Eede, H. Aarts, H.-J. Buhk, G. Corthier, H.J. Flint, W. Hammes, B. Jacobsen, T. Midtvedt, J. van der Vossen, A. von Wright, W. Wackernagel, A. Wilcks. Food and Chemical Toxicology 42 (2004), 1127-1156.

arising from the consumption or use of these products compared to those products of biotechnology approved by the European Communities prior to October 1998?

General comments

786. The European Communities notes that there is general support amongst the independent experts for the rationale for monitoring, and support amongst some for the view that monitoring may be appropriate for unanticipated risks having regard to the state of scientific understanding (noting in particular that it has developed significantly since 1998).

Detailed comments

787. The Panel has received responses to this question from Dr. Nutti, Dr. Snow and Dr. Andow.

788. Dr. Nutti considers that

> "the biotech products at issue in this dispute should be treated in the same way as the biotech products approved by the European Communities prior to 1998. The products approved prior to 1998 are the so called "first generation" products. Most of them had the new trait introduced in order to become insect resistant or herbicide tolerant, and they were assessed as equivalent to their conventional counterparts. The products approved prior to October 1998 have been consumed in different regions of the world (USA, Canada, Argentina, Australia, New Zealand) and, no adverse effects related to their consumption have been reported in any of these countries." Dr. Nutti does not appear to object to monitoring or consider that there is no scientific or technical rationale under certain circumstances: "Continuous use of risk assessments based on the Codex principles and, where appropriate, including post market monitoring, should form the basis for evaluating the safety of GM foods."

789. Dr. Snow notes that this question refers to products for which no significant risks have been identified, whereas many of the questions above refer to products for which one or more Member States *have* identified potential risks. She states that

> "the scientific basis for which environmental consequences to monitor and how to do this in a scientifically rigorous manner are not well developed (e.g., NRC 2002). There are certain types of environmental effects for which monitoring can be justified on scientific or technical grounds. Specific and plausible environmental harms should be identified before allocating time and money to these efforts."

790. In his response Dr. Andow begins by noting that "For all of the products at issue in this dispute, there are some member countries that disagree with the assessment of the relevant SCP (for example) that there are no significant identified risks. This disagreement is sometimes related to different standards of acceptable risk being applied by the disagreeing parties." He considers that this disagreement provides "the only rationale for monitoring potential adverse effects" and that "[i]f all parties agreed that there were no significant identified risks, there would no need to consider monitoring for potential adverse effects." He identifies the various mechanisms by which an unanticipated risk could arise, and states that "All of these possible sources unanticipated effects are equally likely to occur for plant biotech products before 1998 and plant biotech products after 1998." In his view all of these factors "provides a rationale for monitoring".

791. The European Communities notes that none of the responses indicate any *a priori* objection to the principle of monitoring. There is a difference of emphasis, however, as to the circumstances in which it might be justified. Dr. Andow's comments focus on unanticipated risks in a manner which confirms the pertinence of the Community's approach. In light of her other responses, Dr. Snow appears to accept that post-release monitoring will be especially well-justified where pre-release risk assessments have not been carried out as rigorously or as completely as desirable.

792. The Communities considers that these responses are consistent with the approach it has taken, namely that environmental monitoring is required when risk assessments identify potential risks associated with factors such as time and scale of the release (e.g. large scale and delayed impacts) and changes in management associated with cultivation of GMOs. Monitoring will also be justified if the environmental risk assessment identifies gaps in knowledge which are likely to be filled by studies of commercial crops. And monitoring is also used to observe unanticipated effects caused by the cultivation of GM crops such as significant changes in crop production or management practices which may have an environmental impact.

793. Prior to 1998 the extent of knowledge and understanding of potential impacts was less than it is now, so that the approach to environmental risk assessments (ERA) was rather narrowly focused. These earlier approaches have been criticised by some Member States and by Dr. Snow and Dr. Andow. Experience of the methods and approaches to ERA has developed and there is now a greater understanding of complex ecological interactions, allowing consideration of broader environmental issues, even if considerable uncertainties remain. This is the logic which justifies monitoring, in the face of changing scientific knowledge, particularly in relation to environmental risks. This scientific and technical rationale is reflected in the numerous international instruments which endorse the use of monitoring.

Question 100

For those biotech products at issue in this dispute for which an approval has been sought for environmental release (notifications submitted under Directives 90/220 or 2001/18), and for which no significantly different nature or level of risk has been identified, does the information before the Panel provide a scientific or technical rationale for requiring specific agricultural management practices that differ from those for products of biotechnology approved by the European Communities prior to October 1998?

General comments

794. The European Communities notes that there is general support amongst the independent experts for the rationale for requiring specific agricultural management practices, and support amongst some for the view that such practices may be appropriate to address unanticipated risks having regard to the state of scientific understanding (noting in particular that it has developed significantly since 1998).

Detailed comments

795. The panel has received comments from Dr. Snow, Dr. Andow and Dr. Squire. Dr. Nutti considers that this question relates to environmental risks and accordingly does not provide a response.

796. Dr. Snow and Dr. Andow refer to their earlier responses to Question 99. Dr. Squire provides a response that is consistent with the approach taken by the European Communities. He states that

> the innate biological qualities and behaviour of a product may not have changed, but it is quite possible that the context in which that product would operate has changed, in two ways. (A) The physical or agronomic environment is different. The agronomy of these crops changes rapidly: examples of factors that have changed markedly in Europe in recent times are the proportion of autumn-sown crops, the area covered by oilseed rape, and the type and effectiveness of pesticides used. An example, given already, of the change in pesticides is the rise in use of glyphosate as an arable herbicide in some countries in the past few years. (B) The understanding of the GM product's role in the ecosystem has changed. It was also reported previously that some major effects of intensification on arable ecosystems have become well established scientifically only in the last few years. The way arable plants and their food webs are considered is now different from the way they were generally considered in the early 1990s. The question is sometimes referenced back to before the early 1990s: why was the large scale change to autumn-sown cropping in some parts of Europe not scrutinised with the same rigour as the potential change to GM cropping? The answer is that it was thought not to be important, while it is now known to have been very important for farmland food webs.

797. The European Communities agrees with this approach. Since 1998 there is a greater awareness of the impacts that the management of GM crops – especially HT crops - can have on the environment. There has also been a greater desire to ensure that innovations in agriculture are sustainable. Thus there has been a change in attitudes to what is environmentally acceptable in agriculture in the late 1990's, and this has impacted on the evaluation of GMOs in Europe and elsewhere.

798. Beyond the examples given by Dr. Squire, the Communities notes that there are other examples of changes in crop production which may be of importance in this case, including the development of surfaces with no or simplified tillage (due to the increase of useful agricultural acreage per farmer), as well as the significant development of organic farming (which benefits from aids at the national and European Communities levels on the grounds that these farming practices bring environmental benefits). More significantly is the growth in acreage under GM cultivation, giving rise to conditions under which the results of earlier risk assessments may no longer hold true. The scale of use has a significant effect on risk.[286] Some GMHT crops have gained significant spatial scales of use that dwarf those of non-GMHT crops. For example, RoundUp Ready® soybean now occupies >60 of the US soybean area (>20 million hectares), while no non-GMHT trait has come close. With greater scale, small effects can reinforce each other and become more apparent. This would be especially true for non-target effects, such as those found in the farm-scale evaluations carried out in the UK. Relatedly, between 1998 and 2001 new research results were published on spatially explicit models which aim is to simulate the impact of GM crops in cropping systems.[287] This kind of models allows: the ranking of cropping systems according to risk of gene flow (diagnosis); testing the effect of different practices; and determining best crop management to control it.[288] This allows to adapt practices according to risk intensity, technical constraints, spatial distribution of fields in the agricultural landscape e.g. to adapt practices to the diversity of situations.

[286] NRC (National Research Council). (1987). Introduction of recombinant DNA-engineered organisms into the environment: key issues. National Academy Press, Washington, DC

[287] Colbach et al, 2001 a et b.

[288] See Bock et al, 2002, Angevin et al, 2003, Colbach et al, 2004.

Question 101

Does the information before the Panel support the argument that any potential risks from any of the biotech products at issue in this dispute should be mitigated in a manner different than the products of biotechnology approved by the European Communities prior to October 1998? If so, what means of risk mitigation might be envisaged?

General comments

799. The European Communities notes that there is support amongst the independent experts for the view that potential risks from some of the biotech products at issue in this dispute can and should be mitigated in a manner different than the products of biotechnology approved by the European Communities prior to October 1998.

Detailed comments

800. The Panel received responses from Dr. Squire, Dr. Snow and Dr. Andow. These are consistent with the view of the European Communities that developments in scientific knowledge as well as techniques of risk assessment and mitigation mean that new and different approaches to mitigation are required, as compared with the situation which existed in 1998.

801. Dr. Squire's response indicated that there had been a change of approach since 1998: "While products might have very similar qualities, mitigation might differ as a result of new information informing risk or a new perception of the importance of a particular risk. Among ecological topics, the perceived mitigation to reduce the 'severity' of a risk to biodiversity and ecosystem functioning has changed because of a greater appreciation that arable systems have been affected by intense agriculture. Mitigation is unlikely to remain constant. For example, some current mitigation measures which leave margins round the cropped area of land are insufficient when it is appreciated that biodiversity in the cropped areas is necessary to main ecological function there." He added that new research has developed new knowledge: "That risk has not remained the same over time is an inevitable consequence of scientific information on arable systems being collected at increasingly larger scales since the mid-1990s."

802. Dr. Snow's response recognised the possibility that although several of the products that were approved before 1998 are the same as (or similar to) those in the present dispute the possibility could not be excluded that post-1998 scientific knowledge could result in a different approach:

> "The answer to this question hinges on which potential environmental risks might require mitigation, and whether any new scientific knowledge has become available to justify changes to any mitigation plans that were required prior to October 1998. I am not sure which mitigation plans may have been required at that time, but new scientific knowledge that was gained after 1998 certainly could be relevant to risks related to gene flow (see above) and the evolution of resistance in target pests of Bt crops (e.g., NRC 2000)."

803. In a similar vein, Dr. Andow confirms that "there are new risk methodologies and assessment standards being applied to biotech products today than prior to 1998. Thus, it is possible that risks will be identified for new products that were not even considered in the older products. Under such conditions, differences could be justified."

Question 102

Does the information before the Panel support the view that the biotech products at issue in this dispute (including products subject to the member State safeguard measures) give rise to the same types of potential risks to human, plant or animal health or to the environment as novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis? If so, for any biotech product at issue in this dispute are there significant differences, from a scientific perspective, in the nature or magnitude of any potential risks from these products compared to comparable novel non-biotech products taking into account:

- *the specific genetic modification introduced and the resulting product;*
- *the intended use of each product (direct human or animal consumption, further processing for consumption, planting or other use);*
- *any potential risks that may arise from the combination or successive use of biotech products or comparable novel non-biotech products.*

Please explain with reference to specific products at issue in this dispute.

General comments

804. The European Communities notes that there is support amongst the independent experts for the view that the biotech products at issue in this dispute (including products subject to the member State safeguard measures) give rise to different and new types of potential risks to human, plant or animal health or to the environment as compared with novel non-biotech products.

Detailed comments

805. The Panel received responses from Dr. Andow, Dr. Snow and Dr. Nutti. The responses from the first two indicate that biotech products give rise to new risks as compared with non-biotech products. This is consistent with the approach taken by the European Communities in these proceedings.

806. Dr. Andow provided a detailed response on the differences between conventional breeding and "molecular breeding," referring to the differences between clonal species (e.g. potato and banana) and sexual species (e.g. oilseed rape, maize and soybean) and, as regard sexual species, between outcrossing species (e.g. maize and oilseed rape) and inbreeding species (e.g. soybean and wheat). As regards the type of risk, Dr. Andow stated that "there are no differences in the types or kinds of risks posed by biotech crops compared with their non-biotech counterparts. The kinds of risks include toxicity to humans and animals, allergenicity, nutrition, potential for producing disease, gene flow risks, non-target and biodiversity risks, and resistance risks." However, he concludes that "Within these kinds of risk, there are new risks of biotech plants." Dr. Andow states clearly that "Nearly all risks associated with novel toxins (e.g., all Bt crops) introduced into crop plants are new risks", and refers to "other risks of a new nature" identified in responses to other questions. He confirms that the European Communities is correct in considering that new open reading frames (ORFs) "could theoretically produce a new protein, which could cause or influence a new risk" and that insertional mutagenesis is "another outcome of transgenesis that is new to breeding, and could cause or influence a new risk". He clearly rejects Canada's claim that transgenesis allows more precise control than selective breeding. And he concludes that although the US, Canada and Argentina may be correct in saying that translocations and other genomic disruptions can occur in conventional breeding, such

genomic disruptions "are normally rare in conventional breeding". Dr. Andow refers to ongoing research which may lead to technical improvements which could "alter" these concerns.

807. In relation to environmental risks, Dr. Snow adopted a similar approach:

> In my opinion … the biotech products in this dispute could be considered as different from non-biotech products in the some cases". Her response referred in particular to pesticide-producing crop plants and to herbicide-tolerant crop plants with feral (volunteer) populations or wild relatives that can hybridise with the crop.

808. In relation to non-environmental risks, Dr. Nutti stated that: "I am of the opinion that both novel non-biotech products and biotech products should be assessed in a comprehensive, scientific, step by step , case by case bases, so the same safety assessment principles should be applied in all cases." To the extent that this response suggests equivalence of risk it is not consistent with the views of Dr. Andow and Dr. Snow.

809. The European Communities would like to point out to the Panel, however, that there is wide international agreement that each GM product should be assessed on its individual perits, taking into account the individual transformation event and the particular genes that have been introduced. In that respect, potential risks, in particular unintended or non target effects, are certainly specific to each of the biotech products. The European Communities would like to refer to the comments it provided on the evolution and the broader issues of risk analysis of GM products in Section III on general and methodological issues.

810. But more strikingly, this being taken into account, it is noteworthy that most of the products in dispute contained traits and genes that are specific to GM products, as they have not net been found or obtained without the use of genetic engineering techniques (glyphosate, gluphosinate resistance, Bt resistance). Consequently, they present unique protential risks, which may not compare with non GM products.

Question 103

Does the information before the Panel support the view that any of the biotech products at issue in this dispute poses a substantially greater risk as regards the direct or indirect consequences of unintentional "contamination" of other plant varieties than a comparable novel non-biotech products, such as one of the 2300 different crop varieties that have been developed using induced mutagenesis?[289] *If so, what means of risk mitigation might be envisaged?*

General comments

811. The European Communities notes that there is support amongst the independent experts for the view that the biotech products at issue in this dispute may pose a substantially greater risk, in particular as regards indirect consequences of unintentional "contamination".

[289] FAO/IAEA (Food and Agriculture Organization of the United Nations/International Atomic Energy Agency). 2001. FAO/IAEA Mutant Varieties Database. Available online at http://www-infocris.iaea.org/MVD/.

Detailed comments

812. The Panel received responses from Dr. Snow, Dr. Squire, Dr. Andow and Dr. Nutti. These responses indicate scientific support for the view that some of the biotech products at issue in the dispute may pose a substantially greater risk by means of indirect contamination. The extent of the risk will depend on definitions of harm (including economic harm) and the nature and extent of the activity. This confirms the approach adopted by the European Communities.

813. Dr. Snow indicated that "The present dispute involves only a few crop species with a limited number of transgenic traits, and the question is whether the dispersal of these traits to other plants, by means of pollen and seed movements, could pose substantially greater risks as compared to non-GM traits." She stated that some of the biotech products at issue in the dispute posed "risks that are greater in certain GM crops as compared to their non-GM predecessors" (referring to her responses to Part I and answers to Question 6 and others).

814. Dr. Andow considered that risk and "contamination" "depend on the scale of release and the nature of the adverse effect". Risk depends on exposure and adverse effect. He states that "There is little evidence to suggest that *ceteris parabis*, gene flow will be greater from a transgenic variety than a conventional one", but that "There has been little discussion and less agreement over the nature of the adverse effects of contamination". Depending on the definition of harm, "the risk associated with biotech crops is substantially greater than the risk associated with any of the conventionally produced varieties". He suggests that "the probability of cross-contamination rises slowly with spatial scale for very small scale production, but once it reaches a large-size threshold, the probability rises much faster".

815. Dr. Squire concludes that as between certain crops

> "Given present knowledge of the life cycle and reproductive behaviour of the crops, there is no reason to suppose that biotech crops confer different degrees of impurity compared with crops produced from, say, induced mutagenesis."

816. Dr. Nutti's response implies that there is no difference of risk between plants that were developed using induced mutagenesis and those produced from the Recombinant-DNA technology. Unlike other responses, however, and in particular that of Dr. Andow, she provides no indication of the reasoning which might assist the reader form a view as to the basis upon which she reaches such an implied conclusion.

Question 104

From a scientific perspective, is there a significant difference in risks to human, animal or plant health or the environment arising from the use of a bacterial antibiotic resistance marker gene, or part thereof, in any biotech product at issue in this dispute (e.g., Monsanto Bt cotton (531), Monsanto Roundup Ready cotton (RRC1445), Amylogene starch potato) compared to novel non-biotech products, such as comparable plant products produced by selective breeding, cross-breeding and induced mutagenesis?

General comments

817. Consistently with the approach set out above, the European Communities notes that there is support amongst the independent experts for the view that there is a significant difference in risks to human, animal or plant health or the environment arising from the use of a bacterial antibiotic

resistance marker gene in any biotech product at issue, including as regards other non-biotech products.

Detailed comments

818. The Panel has received a response only from Dr. Nutti.

819. Dr. Nutti states that

> "the use of antibiotic resistant marker genes has been recognized as a safe tool and its employment should be evaluated on a case by case basis. The same rule should be applied to novel non-biotech products, such as comparable plant products produced by selective breeding, cross-breeding and induced mutagenesis. I mean, these products should be evaluated on a case by case basis, so the same safety assessment principles should be applied in all cases."

820. In relation to other questions Dr. Nutti has indicated that she has no expertise in environmental risk, so this response must be taken to be limited only to non-environmental risks. Even if, as with other responses she provides no indication of the reasoning which might assist the reader form a view as to the basis upon which she reaches such an implied conclusion, the European Communities strongly disagrees with Dr. Nutti's views that ARMG has been "recognized as a safe tool" for the reasons outlined in its comments to Questions 1, 2 and 98, and in accordance to the relevant latest Codex guidelines which recommend not to use such ARMG. Furthermore, the European Communities notes the apparent inconsistency between Dr. Nutti's response to this question and that of Question 98, where she recognises that "the use of alternative transformation methods, which do not use ARMG, is encouraged".

821. The European Communities refers to its comments under Questions 1 and 2 for further detailed considerations regarding the use of ARMG, or part thereof may pose a significant risk of harm.

822. Furthermore, the European Communities would like to note the potetntial inconsistency of Dr. Nutti's response, when she states that the same rule (namely that the use of antibiotic resistant marker genes has been recognized as a safe tool and its emplyment should be evaluated on a case by case basis) should be applied to novel non-biotech products, such as comparable plant products produced by selective breeding, cross-breeding and induced mutagenesis. Indeed, ARMG are bacterial genes, and although it may not be excluded that such genes may be seldomly found in plant genomes, as nobody has systematically searched for it, they can only be present in the plant biotech products in dispute through genetic modification technique. There does not exist yet any novel non biotech plant product produced by selective breeding, cross-breeding and induced mutagenesis which has been found to contain ARMG, and therefore Dr. Nutti's comparison does not make sense.

Question 105

For those biotech products at issue in this dispute for which no significantly different nature or level of risk has been identified, is there a scientific or technical rationale for monitoring the occurrence of potential adverse effects, or of unintentional effects, arising from the consumption or use of these products compared to novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis? If so, would such monitoring relate to the specific genes or traits introduced into a biotech product, and how would this compare with the monitoring of induced changes in novel non-biotech products?

General comments

823. The European Communities notes that there is support amongst the independent experts for the view that there may be a scientific or technical rationale for monitoring the occurrence of potential adverse effects, or of unintentional effects, arising from the consumption or use of biotech products (as compared to novel non-biotech products) even where no significantly different nature or level of risk has yet been identified, having regard to the possibility of unanticipated consequences.

Detailed comments

824. The Panel received responses from Dr. Snow, Dr. Nutti and Dr. Andow. Taken together these are consistent with the growing support now evident in international instruments and around the world for monitoring of unanticipated effects of biotech products, including through general surveillance schemes, without prejudice to whatever monitoring may be required for non-biotech products. These responses support developments in the European Communities towards greater monitoring in the face of uncertainties.[290]

825. Dr. Snow's response takes the question as stated that "It does not seem logical to require monitoring if no risk has been identified (see Question 99)." (emphasis added). Conversely, it would appear that Dr. Snow accepts that if a risk has been identified it would be logical to require monitoring. Dr. Snow's response cannot be taken as an indication of her view that the products which are the subject of this dispute fall within the category of products for which no significantly different nature or level of risk has been identified. Furthermore, her answer does not address all potential unanticipated and non target effects which have been discussed elsewhere by the experts and in the EC comments, which may indeed require also monitoring and general surveillance programs, even if, by definition, these portential effects have gone undetected at the stage of risk assessment because of limited scientific knowledge.

826. Dr. Nutti states that

> "the monitoring of occurrence of potential adverse effects or of unintended effects should be carried out based on scientific parameters in both cases, that is, for those biotech products at issue in this dispute, for which no significantly different nature or level of risk has been identified, and for novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis. In both cases, I am considering that the products were assessed as safe."

She recognises the difficulty of monitoring for "any effect that we are not aware of" and proposes that the subject should be "introduced in the agenda of the Codex Alimentarius Task Force on Foods Derived from Biotechnology, which will start working in September 2005."

827. Dr. Andow considers that "Monitoring can be used to look for unanticipated effects" and that "monitoring may be partially substitutable for molecular and biochemical characterization". He considers that "Monitoring may also substitute for identifying all possible effects, by covering various categories of unanticipated effects". As regards the objectives of monitoring, he states that monitoring related to a specific potential adverse effect "would have to relate to the specific genes/ traits in the transgenic crop", whereas for unanticipated effects "a more general approach is needed".

[290] See Wilson, Latham and Steinbrecher, 'Genome Scrambling – Myth or Reality? Transformation-Induced Mutations in Transgenic Crop Plants', EcoNexus technical Summary Report, October 2004.

Question 106

For those biotech products at issue in this dispute for which an approval has been sought for environmental release (notifications submitted under Directives 90/220 or 2001/18), and for which no significantly different nature or level of risk has been identified, does the information before the Panel provide any scientific or technical rationale for requiring specific agricultural management practices that differ from those for novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis?

General comments

828. The European Communities notes that there is support amongst the independent experts for the view that there may be a scientific or technical rationale for requiring specific agricultural management practices in respect of biotech products (as compared to novel non-biotech products) even where no significantly different nature or level of risk has yet been identified, having regard to the possibility of unanticipated consequences.

Detailed comments

829. The Panel has received responses from Dr. Squire and Dr. Andow.

830. Dr. Squire states that if no significant level or type of risk has been detected, then no particular change in practice should be needed. However, he recognises that if thresholds are imposed (e.g. 0.9%, specifically for GM varieties in non-GM varieties) "then there will need to be different agricultural practices for those GM varieties that leave volunteers or spread genes by pollen to neighbouring, sexually compatible crops." He also concludes that

> "Under a system of coexistence in which a threshold (GM in non-GM) was imposed, the agricultural practice might well have to change in a crop such as oilseed rape to ensure that threshold would be met. Longer intervals than normal between oilseed rape crops, some regional segregation of GM and non-GM crop types and the dropping of varietal associations (80% male sterile, 20% own pollen) from general use would probably be necessary. Given present knowledge, these changes would be in consequence of an imposed threshold of GM in non-GM product, not of any inherent food-risk in the GM product itself."

831. Dr. Andow concludes that disagreement as to different standards of acceptable risk being applied by the disagreeing parties "provides the only rationale for risk management. If all parties agreed that there were no significant identified risks, there would no need to consider monitoring for potential adverse effects. If only some of the parties recognized the risk, then some kind of conditional risk management could be justified. For example, one condition for the management measures could be the country of use."

832. The European Communities generally agrees with these responses (although it considers that Dr. Squire's response is premised on an assumption that the initial risk assessments were correct in their methodology and outcome (a point which is refuted by Dr. Andow, Dr. Snow and Dr. Squire in some of their earlier responses). The Communities recognises that both conventional and GM crops have shown unintended and undesirable effects after release (e.g. reduced efficacy of pest resistance, boll drop, decreased yields, increased requirements for fertilisers etc), so the screening systems are obviously not always able to detect unintended effects prior to large scale release. However, because of the vastly increased scale of GM crop production relative to most conventional crop counterparts, the Communities considers that post-release monitoring is even more important for large scale

production GM crops than for conventional crops grown on smaller scales (because the extent of any harm would be much greater due to scaling effects). This approach is supported by the responses to the Panel.

Question 107

Does the information before the Panel support the view that the biotech products at issue in this dispute (including products subject to the member State safeguard measures) give rise to the same types of potential risks to human, plant or animal health or to the environment as foods produced using biotech processing aids, including yeasts, bacteria or enzymes that have been modified using recombinant DNA technology? If so, for any biotech product at issue in this dispute are there significant differences, from a scientific perspective, in the nature or magnitude of any potential risks from the products at issue in this dispute compared to foods produced with biotech processing aids, taking into account the intended use of each product (direct human or animal consumption, further processing for consumption, release into the environment, any other use).

General comments

833. The European Communities notes that there is support amongst the independent experts for the view that the biotech products at issue in this dispute (including products subject to the member State safeguard measures) may give rise to potential risks to human, plant or animal health or to the environment, if the food is made available to the environment, although the nature and extent of that risk may be lower.

Detailed comments

834. The Panel has received responses from Dr. Andow and Dr. Nutti.

835. Dr. Andow expresses the opinion that since in general, foods produced with biotech processing aids "are not expected to be able to self-reproduce in the environment", so that the kinds of risk associated with these foods "do not include any of the environmental risks associated with transgenic plants." However, he also believes that foods produced with biotech processing aids "can have some non-target effects if the food is available in the environment" and that these effects "are more similar to the effects of chemicals rather than the effects of living biological organisms." In respect of these products unanticipated effects could arise from a transgene by a number of mechanisms, although in each case "the likelihood of it occurring in a transgenic bacterium or yeast (that is typically used as a food processing aids) is much lower than in a transgenic plant."

836. Dr. Nutti addresses only non-environmental risks. In her view "the safety of the new microorganisms and of the product thereof should be assessed according to the *FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Microorganisms."*

837. The European Communities generally agrees with the approach taken by Dr. Andow. As regards environmental issues, the modified food product is non-viable and the environmental impacts of the transgenes would be restricted to wildlife and environments exposed to these food products or the GM processing aids. Risks associated with these products would be case specific depending on the traits produced by the processing aid, the interaction with the crop raw material and levels of environmental, human and animal exposure. Since the processing is largely done in contained facilities (fermenters etc) the risk of environmental exposure will generally be low. However, gene products may pass directly into the environment from human activity or factory discharges or may

pass indirectly into the environment after consumption by humans and animals. Release of the GM bacteria or yeasts which are the source of processing aid could have some environmental impacts depending on the species of organism and the trait. If they can survive as free living organisms in soil or water and/or mate with free living microorganisms then there may be some ecological impacts which would need to be evaluated.

Question 108

Is there a significant difference in risks to human, animal or plant health or the environment arising from the use of a bacterial antibiotic resistance marker gene, or part thereof, in any biotech product at issue in this dispute (e.g., Monsanto Bt cotton (531), Monsanto Roundup Ready cotton (RRC1445), Amylogene starch potato) compared to foods produced with biotech processing aids, including yeasts, bacteria or enzymes that have been modified using recombinant DNA technology?

General comments

838. The Panel has received a response from Dr. Nutti. It raises the same points as arise in questions 1, 2, 98 and 104, and the European Communities refers to its comments thereunder.

Question 109

For those biotech products at issue in this dispute for which no significantly different nature or level of risk has been identified, does the information before the Panel provide a scientific or technical rationale for monitoring the occurrence of potential adverse effects, or of unintentional effects, arising from the consumption or use of these products compared to foods produced with biotech processing aids? Does the information before the Panel provide a scientific or technical rationale for mitigating any potential risks arising from the biotech products at issue in this dispute in a manner differently than products produced with biotech processing aids?

General comments

839. The European Communities notes that there is support amongst the independent experts for the view there is a scientific or technical rationale for monitoring the occurrence of potential adverse effects, or of unintentional effects, arising from the consumption or use of biotech products compared to foods produced with biotech processing aids, even where no significantly different nature or level of risk has yet been identified, having regard inter alia to unanticipated effect, and that monitoring may be justified.

Detailed comments

840. The Panel has received responses from Dr. Andow and Dr. Nutti.

841. Dr. Andow states that sources of sources unanticipated effects

> are more likely to occur for plant biotech products than for foods produced with biotech processing aids. This provides a rationale for monitoring, but no rationale for mitigation, as there are no concrete effects that can be mitigated.

842. Dr. Nutti states that

> For those biotech products at issue in this dispute for which no significantly different nature or level of risk has been identified and for foods produced with biotech

> processing aids, where the processing aid and the Recombinant-DNA microorganism have been assessed as safe, I am of the opinion that the monitoring of occurrence of potential adverse effects or of unintended effects, in both cases, should be conducted based on scientific parameters.

843. The European Communities notes that neither expert expresses an objection in principle to monitoring, even where no significantly different nature or level of risk has been identified, and that Dr. Andow has identified a positive rationale on the basis of unanticipated effects. As regards Dr. Nutti's approach, based on a symmetry between both types of products, the European Communities considers that this may apply to GM yeasts and bacteria based on Codex guidelines for their safety assessment but that it does not apply to processing aids consisting of GM enzymes, based on the considerations of the different nature, function, dissemination, viability, activity, complexity, and consumer exposure of the GM products at issue.

VIII. THE PANEL'S ADDITIONAL QUESTIONS

Question 111

Please provide an assessment of the US statements regarding the evaluation of the safety of biotech products in paragraphs 128-133 of the US Supplementary Rebuttal Submission, and in particular of the statement in the last sentence of paragraph 133 that: "... where all of the data consistently provide no indication of adverse effects, and there is no specific indication that the data submitted are inadequate, there is generally no reason to expect that any remaining risk as gone undetected, and that further studies are warranted". What relevance does the foreseen end product use(s) have in the context of the evaluation of the safety of that product?

General comments

844. With question 111, the Panel's sought advice on the statements in paragraphs 128-133 of the US supplementary rebuttal submission, where the US describes its approach to assess the risk of biotech products, and in particular, on the sweeping statement of the US, in paragraph 133, which seeks to minimize the amount of information to be supplied by an applicant, omitting the difficulties and the safety problems that may arise when following the US federal approach.

845. Dr. Nutti and Dr. Snow both answered that question succinctly, but with different approaches and emphasis; Dr. Snow addressed certain environmental concerns and flaws of the proposed US approach, while Dr. Nutti presented her own approach to food safety assessment. Both, again with different emphasis, not only agree with the relevance of end products uses in risk assessment, but also consider it necessary in the assessment.

846. The European Communities agrees with Dr. Snow when she concludes that

> "The last sentence of paragraph 133 does not acknowledge that important scientific information about environmental consequences could be lacking in specific cases, in which case further scientific studies would be warranted"

847. The European Communities believes that that statement is equally applicable to other aspects of the risk assessment, such as food or feed safety, and, as Dr. Snow herself notes, the inherent problems and shortcuts of the US proposed approach generally apply to the products in dispute, where further information was sought by one or the other authority, in order to complete its risk assessment in accordance with the lack of important safety information.

848. The issues raised by the Panel's question, albeit of a general nature, are very important as they are issues of principle for conducting a risk assessment, applicable throughout all products in dispute and their respective applications, when considering whether there was a delay, and, if so, whether it was undue.

849. Therefore, because of the succinct character of both replies, which didn't cover all necessary aspects of the inappropriateness of the US approach, and of the general inadequacy of Dr. Nutti's reply as regards food safety, the European Communities will comment in detail below and provide its own analysis. It has prepared this information with the assistance of independent scientists, experts in this field, and presents it as objectively as possible so as to complete the information available to the Panel.

850. It will explain why the precise requirements *and* the principles for the *ad hoc* risk assessment of a GMO have got to be decided on a case-by-case, in light of the specific information supplied (both its content, and its quality), but also taking into account the likelihood of unintended effects and the particular geographical and environmental conditions of the foreseen intended or unintended uses.

851. As widely explained by the experts replies and the comments submitted by the European Communities, there was and still is a vast amount of open and unresolved issues in the assessment of the biotech products in dispute. These uncertainties warrant the call for certainly more studies than advocated by the US, in order to conduct a proper science-based risk assessment, and to reach a rigorous conclusion, with a reasonable level of certainty, as regards the safety of the biotech products in dispute.

852. The European would like, in this context, to refer the Panel to its general comments in section III of this submission, on general and methodological issues, which are extremely relevant here, in particular on case by case assessments, systemic issues, evolving science, scientific evidence of risks or absence of risks, absence of agreed scientific criteria, judging when the scientific information is sufficient, interpretation of scientific information, surveillance and food safety, and on new developments of broader risk assessment concepts for genetically modified products.

Detailed comments

853. The US statements essentially describes its views as regards, not the conduct of a risk assessment, but the endpoints of information and data requirements necessary for each isolated and independent component of the risk assessment it describes (the safety of the new substance, comparisons made with conventional products, molecular characterization, stability of the modification, field trials analysis, and environmental impacts). The difficulty is not as much the risk assessment paradigm itself, as described, but rather the studies claimed to be warranted by the US to implement each step in this paradigm.

854. The approach presented by the US seems to be essentially inspired by food safety considerations, in light of the little emphasis given to environmental and non target effects. The US risk assessment paradigm is more or less roughly similar to the one used anywhere as regards food safety, even if there is intense debate worldwide as to how to test each of the individual step, and what further components and methods may be necessary to improve it, for instance to better integrate the different steps of the risk assessment, or to assess the GM plant or the GM food as a whole, in its real environment or in its real uses.

855. The US approach is based on the assumption that it is necessary to benchmark the assessment of biotech products against the approaches to assess "conventional" products and techniques, including the most unpredictable plant breeding technology, with old risk assessment techniques,

mainly developed to test the safety and eco-toxicology of individual compounds in the chemical industry.

856. This assumption was the one initially advocated by the industry and became the basis for the regulation of transgenic crops in the US (see for instance, for the FDA, [291]). It hasn't changed since, despite the large number of new issues and the many weaknesses of the US approach, as largely explained by the experts. Each will be addressed below.

857. It is noteworthy that the inherent shortcuts and limitations of the US federal approach to the risk assessment of genetically engineered products have forced the US federal authorities themselves to review frequently their approach in recent years, and sometimes to improve coordination or revise it, albeit scarcely and unwillingly, in the light of many identified flaws and recommendations from its own advisory structures,[292] even from the least progressive ones.

858. Even so, the last paragraph of the US statement is qualified (emphasis added):

> … where *all* of the data *consistently* provide no indication of adverse effects, and there is no *specific indication that the data submitted are inadequate*, there is *generally* no reason to expect that any remaining risk has gone undetected, and that further studies are warranted.

859. As has been indicated in the comments under issue 1, there was almost systematically, in the biotech product applications at stake, missing data, inconsistency of the data provided, inadequacy or bad quality of the data submitted, or specificities of the product or of its intended or unintended uses, which all warranted, according to this very US statement, the requests for further studies.

860. Dr. Snow, in her comments, indicates the lack of relevance of the tests proposed by the US to assess some environmental risks, such as out-crossing impacts. The European Communities would add that this applies in particular to unintended effects and non target organisms. Furthermore, on top of the lack of identification by the US of the methods that could be used as regards "the effects on other organisms in the ecosystem", that could include "indirect impacts", it is doubtful, in light of the succinct methods proposed for food safety, that the US would address multi trophic (ie involving a chain of organisms in complex food webs), sub chronic, or delayed effects of the organisms in its natural environment. Dr. Andow, although not replying to this question, has provided a ample evidence to support such a view.

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[291] Kessler DA, Taylor MR, Maryanski JH, Flamm EL, Kahl LS (1992) The safety of foods developed by biotechnology. Science 256: 1747-1832.

[292] See for instance the many findings and recommendations regarding the analysis of the US APHIS policy and assessment, of GMO monitoring, and of future approaches, stated by the US Committee on Environmental Impacts Associated with Commercialization of Transgenic Plants (Environmental Effects of Transgenic Plants – the scope and adequacy of regulation; 2002; National Research Council; National Academy Press, Washington, D.C.; 320pp.), or the advise given to the US EPA by a FIFRA expert panel, in order to address in its assessment approach for Bt cotton, in particular, unintended effects of GMO (SAP Report No. 2004-05. MINUTES of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) Meeting, June 8-10, 2004, Arlington, Virginia. *A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding*: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management For *Bacillus thuringiensis (Bt)* Cotton Products. 84 pages.), or the advise given recently to the US FDA to revise in depth its approach to the food safety assessment of transgenic products.

861. Dr. Nutti addresses only the toxicological and allergenicity assessment of the food safety, not even of the whole plant, but of the newly expressed character (mainly the protein). In doing so, while quoting the relevant paragraph of the recent Codex guidelines (CAC/GL 45-2003), she presents what tests, in her view, would satisfy these guidelines. She concludes in general terms that if these few tests are satisfactory, no risk is to be expected, which by and large concurs with the US statements scrutinized. She does not address feed safety, animal health, plant health, environmental safety or agro-environmental impacts, which are obviously all part of a necessary risk assessment.

862. While it is clear that her analysis on risks should be dismissed on the grounds of critically missing elements on other aspects than food safety, it is also substantially misleading on the latter.

863. First, she does not address the other important safety aspects referred to in the above-mentioned Codex guidelines, namely the evaluation of metabolites, food processing, nutritional safety, accumulation of other potentially hazardous substances (e.g. pesticides), or antibiotic resistance marker genes. These all have bearing on the safety of foods and may require further testing, as shown below.

864. Second, she does not at all take into account that the assessment of the newly expressed substance requires, by definition, its identification. For that, as an imperative pre-condition, a perfect molecular characterisation is required to fulfil this initial test of the toxicity assessment. As has been shown before, she has often, in specific product applications, dismissed request for further food safety information, when in most cases the molecular characterisation of the GMO has left substantial doubts as regards the integrated construct and/or the expressed genes, hence on the newly expressed substance(s), and not to say anything about unintended genetic changes at, or near, the site of integration of the modification. According to her own words, these doubts about the safety of the new substances remaining would have warranted the use of toxicology or other studies.

865. The points of view of the US and Dr. Nutti are not fully comprehensive, as they imply that the safety assessment is static by not taking into account that our knowledge about biotechnology and safety issues is still evolving. For example, a DNA sequencing study revealed in 2000 that the insert in GM herbicide-resistant soybean, which had already been approved for marketing, contained additional fragments of "foreign DNA". For the safety assessment of the additional insertions, among others, the applicant (Monsanto[293]) argued that these additional fragments could not pose a risk, since no adverse effects had been observed in previously published animal feeding studies with whole feed products derived from Roundup ready soybeans. Had the applicant only carried out the compositional and toxicity studies mentioned by the US and Dr. Nutti (and thus not the aforementioned animal studies), no data would have been available to judge the safety of these additional, previously unsuspected modifications.

866. The US statements call for assessing the "stability of the transferred genetic material and the demonstration of the Mendelian inheritance of the introduced genetic material", as important safety considerations. Once done, it would be a static assessment, as presented by the US. However, more and more often, it is apparent that many GM crops that have been previously assessed for the stability of the genetic modification appear to have undergone significant changes in their genetic modification structure. This does not come as a surprise, as the plant genome is not a fixed object, and the plant has to adapt to the presence of the newly inserted construct, but it raises important safety questions if the initial assessment is not properly reviewed.

[293] Monsanto (2000) Updated Molecular Characterization and Safety Assessment of Roundup Ready Soybean Event 40-3-2. Monsanto Co. St. Louis, 20 pp.
http://archive.food.gov.uk/pdf_files/acnfp/summary.pdf

867. Third, as regards the toxicity and allergenicity studies she advocates, she only supports the use of acute toxicity on mice (to assess the acute toxicity of the newly expressed protein only), and very limited investigation of allergenicity potential. Not only, as regards the allergenic potential which can not be assessed with certainty, does this not align with the most recent views and methods to assess allergenicity, (see also the annex to the Codex guidelines she cites), but she also does not mention the use of chronic tests and other tests, which may often be necessary to assess acute *and chronic and sub-chronic* toxicity of new substances with no known history of safe food use. Examples of relevant tests are mentioned in paragraph 39 of the mentioned Codex guidelines.

868. Another example of evolution of knowledge that is relevant for safety assessment is the assessment of potential allergenicity based on computer-aided comparison of the structures of transgenic "foreign" proteins with the structures of allergens. The knowledge on structures of allergens is still growing and new allergens are identified. Therefore, it is not unlikely that a protein at present may not show a relevant similarity to known allergens, but will do so in the future.

869. There is an obvious discrepancy in the allergenicity assessment proposed by the US and by Dr. Nutti with other relevant views. The US and Dr. Nutti assess the potential allergenicity of a product with the focus of newly introduced proteins, in line with their endorsed principle of substantial equivalenve, without caring for possible unintended effects and the need for a molecular characterisation. From 1996 to now it became gradually more evident that insertion of constructs into plant genomes often causes unintended effects, especially at the recombination boarders/ flanking region of insertion. Because of these changes in open reading frame (the segment of DNA coding fro protein), expression of new or altered proteins is possible. These consequences of molecular events need to be assessed by the molecular characterisation in combination with allergenicity testing of the potentially new expressed /changed proteins, or where this is not sufficiently clearly defined, by assessment of the whole product. Dr. Nutti doses not seem to adhere to this approach.

870. There has also been discrepancy between experts in general about allergenicity testing schemes. Even the present strategy based on a sequence prediction model established by FAO/WHO was contradicted by important groups[294]. Difficulties raised by the current approach are summarised in Jank and Haslberger, 2003[295]. Whereas a general decision tree is generally accepted many experts consider it often sufficient to do sequence homology search and stability testing, whereas some experts also in some of these cases ask for more testing. Also the ways to perform the sequence homology search is under dispute. This idea is not fully explained in CODEX but there are elements in it and the latest FAO/WHO expert consultations address it more clearly (CAC/ GL 45-2003 and its annex; Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2001).

871. The latest FAO/WHO consultation on GM foods (2003) agrees that "It has been recognized that there is no single parameter that can predict the allergenic potential of a substance. A strategy to assess allergenicity of biotechnology products has been formulated (FAO/WHO, 2001; Codex Alimentarius Commission, 2003), which relies on the parameters: source of the gene, sequence homology, serum testing of patients known to be allergenic to the source organism or to sources distantly related, pepsin resistance, the prevalence of the trait and assessment using animal models. The Consultation recommended that additional efforts should be directed to the further development and validation of models and that there is a need to improve the accessibility and interconnectivity of

[294] Stadler and Stadler, Allergenicity prediction by protein sequence, FASEB, J 2003, 17, 9, 1141-3

[295] Jank and Haslberger, 2003. Improved evaluation of potential allergens in GM food, Trends Biotechnol., 2003, 21, 6, 249-250)

existing databases or to establish a centralized database on allergenic linear and conformational epitopes and tools for screening transgenes for allergenic potential.

872. Fourth, Dr. Nutti does not refer to the fact that the toxicology of the newly expressed proteins in the GM products at stake, was often tested with "surrogate" proteins (ie isolated from heterologous systems, different from the GM plant, see review by Freese and Schubert (2004))[296], without proper demonstration of biochemical, structural, or functional equivalence of the surrogate protein to its counterpart (for instance as regards mutational changes, post translational modifications, or others), as recommended in Paragraph 40 of the Codex guidelines.

873. Fifth, she states that exposure to human diet that has no indication of adverse effects would be a criteria to take into account. Notwithstanding the ethical question of performing pre marketing toxicity tests on humans, that statement is not scientifically sound for anything else than acute toxicological risk, as explained by the European Communities in the section of general and methodological issues, on surveillance and food safety. Standard epidemiology says that, in the absence of exposure data with respect to chronic conditions, there is simply no way of ascertaining any effect – or lack thereof – on human health.

874. Sixth, she refers to properly conducted substantial equivalence tests, while it is clear from the US proposed methods, that these would be limited to key components; for example, carbohydrates, proteins, and fatty acids, amino acid and vitamin content, as well as naturally-occurring toxicants, antinutrients, and allergens. Substantial equivalence as applied in such a way has received significant criticism[297], including within the relevant Codex work, as it would obviously miss important changes for the safety of the product.

875. The safety assessment of selected compounds and the "targeted" compositional analysis, also on selected compounds {e.g. Codex alimentarius; paragraph 16}, leaves room for "unpredictable" unintended effects, whose likelihood will be greater in GM crops with complex or major modifications {e.g. Codex alimentarius; paragraph 15}.

876. The difficulties with substantial equivalence is at least three fold: (i) it may largely overlook significant and numerous differences in composition that may remain undetected, (ii) it is often used as an endpoint, rather than a starting point of the risk assessment, and (iii) it is a essentially only a series of chemical tests, which establish gross differences or similarities (but within the broad range of values for that species, including the less safe ones as regards toxicants or antinutrients), but by no means able to test or demonstrate biological effects[298], which would be the most relevant criteria.

877. Very large number of changes of endogenous metabolites may occur as a consequence of the genetic modification. Such unintended changes in GM crops could be detected by metabolic profiling[299], but would remain silent according to a standard substantial equivalence test, as proposed by the US. For instance, Roessner et al. (2001)[300] have detected by metabolic profiling a very large amount of significant unintended changes in GM potato, many more than previously assumed. 9 novel

[296] Freeze W & Schubert D (2004). Safety testing and regulation of genetically engineered foods. *Biotechnology and Genetic Reviews*. **21**: 299-324.

[297] See relevant references in the comments on substantial equivalence in section III on general and methodological issues.

[298] The brain from a mad cow showing advanced spongiform encephalopathy would pass a test of substantial equivalence undetected!

[299] A relatively new technique which looks at a wider range of plant chemicals than before

[300] Metabolic Profiling Allows Comprehensive Phenotyping of Genetically or Environmentally Modified Plant Systems, Ute Roessner, Alexander Luedemann, Doreen Brust, Oliver Fiehn, Thomas Linke, Lothar Willmitzer, and Alisdair R. Fernie; The Plant Cell, Vol. 13, 11–29, January 2001.

compounds were detected in GM potato tubers not found in the control plants, and more than half of the 88 plant chemicals measured had been altered (levels) as a consequence of the genetic modification.

878. It is not clear how significant these results are, but it seems to be a striking indication of how some genetic modifications can cause widespread alterations in cellular metabolism. If Roessner et al has measured a representative sample of all potato compounds, the proportion of all unmeasured potato compounds whose level could be altered could be anything like the "over half" found in this study. If 5,000 is typical estimate of the number of different molecules for a species, one could be talking significant alterations in the expression levels of hundreds of genes, plus dozens or hundreds of novel compounds. It might be assumed that most of these changes would likely not have any adverse impacts, but it would still be a very large pool of alterations, that, it seems, with regards to the novelty in the plant genome of the newly introduced character, could bring safety concerns, hence the need for further testing methods.

879. In literature, other ways to detect unintended effects than whole feed studies on animals are described, such as the use of advanced analytical technologies called "profiling" or "metabolomics". These techniques are in development, but have not been validated yet for routine application in the safety assessment of GM foods {reviewed by Chassy et al., 2004[301]}. These new techniques (metabolic profiling) are helping to confirm many scientists' historical views that genetic modification does not just result in addition of one gene with no reason to expect any further changes that could result in potential risks (the 'clean technology' paradigm). This type of research has general implications for food and feed safety, but also for the environment (target and non-target organisms could be affected in unpredictable ways); it further supports case-by-case assessments, as each GM event could be uniquely different to the parental or isogenic line (the closest non-GM line that the Codex recommends to use as a control). This type of new knowledge also seriously questions and undermines the safety approach advocated the US as regards food safety.

880. Seventh, Dr. Nutti does not consider feed safety concerns. As explained in section III on general and methodological issues, there is little guidance as regards feed safety testing. The lack of identification of human safety concern does not necessarily correlate with a lack of safety concern for target animal of the feed derived from the GM plant (or even less for non target animal), as physiology, metabolism, plant parts consumed, processing and exposure can each and all be different, in particular for non mammal target species. In the light of the requirement to take into account the end use of the GM product, feeding studies on target animals would be highly recommended for feed uses, and have rarely been provided.

881. For instance, there has been reports[302] of significant changes in the lignin content of some Bt maize; while this may have limited or no bearing for the safety of the processed product in food (and may also have gone undetected following the safety approach proposed by the US and Dr. Nutti), it could have significant nutritional, if not toxicological, impact on target animals and on wild animals exposed to the maize in the field, including soil organisms, which would warrant further testing.

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[301] Chassy, B., Hlywka, J.J.,. Kleter, G.A., Kok, E.J., Kuiper, H.A., McGloughlin, M., Munro, I.C., Phipps, R.H., Re, E.B., Reid, J.E. (2004) Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology, Prepared by a Task Force of the ILSI International Food Biotechnology Committee. Comprehensive Reviews in Food Science and Food Safety 3: 35-104.
http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

[302] See for instance Saxena D. and Stotzky G. (2001). Bt corn has a higher lignin content than non-Bt corn. American Journal of Botany. 88(9): 1704-1706.

882. Finally, Dr. Nutti, as the US, dismisses by her approach the likelihood of significant impacts from genome-wide or physiological unintended changes. There are however several reports of such wide-genome induced changes. A recent EcoNexus technical report[303], although it has not been published as a peer review publication, very seriously and even-handedly presents the identification of insertion-site and genome-wide mutations created by plant transformation procedures as "potentially major, but poorly understood" sources of hazard associated with the production and use of GM crops.

883. The significant potential for genome-wide and transformation specific unintended effects were recognised[304] in a recent FAO/WHO (2003) expert consultation on the food safety of GM animals, or in a recent FIFRA expert panel report (see attached[305]).

884. That latest FAO/WHO expert consultation on GM animals updated the entire field of assessing the food safety of GM products (FAO/WHO expert consultations are usually the basis for CODEX recommendations) and concluded that "An extensive molecular characterization of the inserted genetic material construct will generally be required, both before and after the insertional event. The molecular characterization should furthermore comprise an analysis of the copy number and a sequence analysis of the flanking regions of the place of insertion in order to identify any unintended effects. It is recommended that the approach for the molecular characterization should be further standardized to include the flanking regions"

885. This EcoNexus report and the other expert advice available clearly calls for radical changes in the safety approach proposed by the US. There are three basic reasons why one can arrive at the conclusion that there is "no evidence" of any remaining risk: (i) one did not look; (ii) one did look, but the investigations were designed in such a way that they can not find the answer; (iii) on e did look, with appropriate and well designed experiments, with negative results. Unfortunately, with all the difficulties inherent to the need to test the safety of whole organisms "in real situations", because of the novelty of the introduced characters, the majority of the potential questions relating to human health come under (i): we did not look. And most of the science available to the regulators to assess these products, because of lack of proper criteria, harmonised and scientifically sound methodologies to perform these new safety tests, fall under (ii), i.e. they are often poorly designed and inconclusive, and very little comes under (iii).

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[303] See for example: A. Wilson et al. 2004. Genome scrambling – myth or reality; transgenic-induced mutations in transgenic crop plants. Econexus technical report (October 2004). Available at www.econexus.info.

[304] Introduction of a transgene into an animal is not a precisely controlled process, and can result in a variety of outcomes regarding integration, expression and stability of the transgene in the host. The desired outcome generally is stable integration of a single copy of the transgene into a single location in the genome, and not in a functional gene or a regulatory element. However, other outcomes are frequently observed, including integration of multiple copies of the transgene at one locus or insertion of the transgene at multiple locations in the genome. Insertion of the transgene into a host gene may turn the host gene off, sometimes affecting the viability or health of the host. Insertion of a transgene sometimes can affect expression of another gene(s). A transgene may become rearranged before integration, thereby becoming non-functional. During the process of transgenesis, undesired DNA sequences may become inserted into the genome, such as marker genes or selectable markers from the expression vector or contaminating bacterial DNA left over from vector production. Hazards stemming from insertional events or genetic instability can be identified by screening and managed by culling individuals that have undesired events during the course of development of the transgenic line. Expression of the transgene ideally should have no undesired effects on the expression of other host genes or health of the host. Other outcomes, however, have been observed. The transgene can be silenced by methylation or through other mechanisms.

[305] SAP Report No. 2004-05. MINUTES of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) Meeting, June 8-10, 2004, Arlington, Virginia. *A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding*: Product Characterization, Human Health Risk, Ecological Risk, And Insect Resistance Management For *Bacillus thuringiensis (Bt)* Cotton Products. 84 pages

886. The approach advocated by the US is essentially designed to look the one new substance, enzyme, or protein. But it should be clear now to the Panel that plants, tissues, and products derived from GM crops are in many instances more complex than purified enzymes, or proteins. Therefore, additional issues, like for instance the occurrence of unintended changes in the GM crop likely deserve greater attention than the single new protein.

887. The methodological difficulties arising from missing, emerging or new tests to assess such a wide area of complex and uncertain organism-wide potential risks, leaves the assessor and the scientist alike to decide, subjectively, what is the end point of data requirement and risk assessment, and what constitutes a reasonable, science-based, evidence of lack of risks.

888. A last issue, although not explicitly apparent in the US statements on methods to assess safety, merits consideration: the issue of whether and how to assess the genetic modification stacking (an hybrid between two individual GM parents carrying the double modification). Following the US and Dr. Nutti's approach, there would be no need to assess any new hybrid, as all individual components would have been assessed separately (each new gene products and each parent). And indeed, the US deregulates GM crops, which can then be bred without any further assessment.

889. The EPA FIFRA expert panel report, previously mentioned, addresses this issue and concludes as follow:

> In principle, traditionally bred transformed lines should have the same characteristics as the parent organisms. However, analyses of events have pointed to potential different outcomes in some cases where the basis of these events is not presently fully understood. Two specific issues for consideration are:
>
> (1) A molecular characterization of the new product should show that the recombinant traits/ sequences in the new product are identical to the insertion /traits in their parental lines. In principle, the method of breeding the two parental lines could affect the genetic characteristics of the inserted traits. Therefore, evidence is needed that no such effect has occurred. For these products, a risk assessment combining evidence from parental lines and the product should be requested.
>
> (2) Stability is another concern. For other similar products, it is uncertain how many generations need to be observed to establish stability of inserted traits.

890. Consistent findings were made in the report on adequacy of the [US APHIS] regulation of transgenic plants, by the US Committee on Environmental Impacts Associated with Commercialisation of Transgenic Plants, and published by the National Research Council and mentioned earlier:

> Finding 7.5: The current APHIS approach for deregulation does not assess the environmental effects of stacked genes for non additive or synergistic effects on the expression of individual genes, nor does it assess stacked genes for cumulative environmental effects at the field level.
>
> Finding 7.6: There are at least two levels at which scientists and regulators must look for interactions between inserted genes with regard to environmental effects: (1) the individual plant phenotype and (2) the whole-field or farming system level.

891. Finally, as regards the use and validity of field tests data, there is much to debate, as the validity and conditions of the tests, and their relevance for other regions and different agro-environmental conditions, may lead to significant request for further risk assessment information. These may as well relate to important safety questions, such as, for instance, the different value of data collected on GM HT crop sprayed with the herbicide or not.

892. The debated question of the relative hazard posed between conventional products and genetically engineered ones will certainly remain an open question after this case; however, it is clear that old, reductionist risk assessment approaches, are found inadequate to assess GM crops, by more and more scientists, and more and more globally, in particular to assess their agro-environmental direct and indirect effects, their non target impacts, and their unintended characteristics, including genome-wide potential changes and their associated possible health or environmental effects.

893. In the light of the expert advice and the above, it is clear that, under today's best science-based understanding, the proposed US approach is flawed, incomplete and, by all means, an unacceptable standard to properly assess the risks of GMOs.

Question 112

Please provide an assessment of the statements regarding chronic toxicity testing in paragraph 43 of Canada's Third Submission and paragraphs 134-138 of the US Supplementary Rebuttal Submission. What relevance does the foreseen end product use(s) have in the context of the evaluation of the toxicity of that product?

General comments

894. The Panel sought advice on the relevance of the Canadian and US assertions referred to in the question. Canada's text does not refer to a particular scientific argumentation and is essentially a legal argument to justify that EC's approval procedures are SPS measures, and hence is not to be commented in this submission. The US text is essentially an illustration, of the disconnected risk assessment approaches, applied respectively to the novel protein and to the substantial equivalence of the GM crop, and as outlined in the US methodology presented in the previous question.

895. Only Dr. Nutti has replied, simply repeating more briefly her line of argument in her previous reply, and dismissing that such tests might be necessary. The European Communities disagree with her statement.

Detailed comments

896. The European Communities will refer the Panel to its detailed comments on the previous question and in section III on general and methodological issues, where there is ample scientific explanation why these studies may be justified. May it simply say here that there are many reasons why chronic or sub chronic studies may be required, including for instance when the inserted construct has been not appropriately characterised and there might be a doubt on the exact identity/modification of the expressed protein, or if previous acute toxicity tests had been performed with surrogate proteins, and the equivalence of the proteins is not sufficiently proven, as requested by the Codex guidelines (CAC/GL 45-2003).

897. The Codex guidelines, in paragraph 37, clearly states that the use of appropriate conventional toxicology or other studies on the new substance may be necessary if, taking into account its function and exposure, doubts about the safety of the new substance remain. This is certainly the case when the

construct is poorly characterised, potential unintended effects of the genetic modification, or when there are doubts on the acute gavage studies (protein used, methodology, interpretation, …).

898. There are generally no accepted clear distinctions available between classic (long-, short term, chronic toxicology studies as developed for testing of pure proteins, pharmaceuticals or chemicals) and whole food toxicology studies (new, and often confused) and feeding studies (developed often for testing of feeds). Furthermore, acute toxicity on mammals would only not assess the toxicity on other animals, which may react very differently and be very important for the assessment of the environmental impact (eg birds, fishes, insects, ..).

899. Acute toxicity testing may be highly inappropriate to find unintended effects because of its lack of sensitivity. Chronic toxicity testing has been proposed by many scientific reports as a tool (together with other profiling methods such as gene- expression micro arrays, biochemical, or proteomic screening). However limitations of whole food studies have been identified and discussed extensively.

900. In fact, many CA authorities started to ask for these studies because of uncertainties in the molecular characterisation. The EU research project on Entransfood has looked at it, and there is agreement for the usefulness of chronic toxicity studies, however some disagreement on their length.

901. Finally, the US EPA FIFRA expert panel mentioned earlier recommends multi year non vertebrate studies for environmental risk assessment. It states that:

> "The Panel found that testing of multi-year field effects on non-target vertebrates is generally essential. It can readily be seen from the numerous publications Brewer et al. (1989, 1989a, 1990, 1992; Tank et al. 1992, 1992a) that significant annual differences are apparent with the same pesticide treatments by the same applicators in the same fields"

902. Although realising the limitations of such studies, chronic toxicology studies are needed especially in the screening for unintended effects of GMOs because there are presently no better methods sufficiently developed.

Question 113

Please provide an assessment of the statements regarding the purpose and use of whole food studies in paragraphs 142-144 of the US Supplemental Rebuttal Submission. What relevance does the foreseen end product use(s) have in the context of using whole food studies in the evaluation of the safety of that product?

General comments

903. The Panel sought advice on the relevance of the US assertion referred to in the question. The US stated that whole food studies were not warranted as a matter of principle, dismissing both a case by case approach, and taking into account the final use of the product, and the target animal with which such studies may have been requested.

904. Only Dr. Nutti replies to this question, essentially repeating the US arguments, that requests for whole food studies in different species does not have scientific support. The European Communities notes that her reply to this question contradicts her reply to question 112, as she stated here that the request for whole food studies was not supported by Science, on the basis of availability

of 90 days oral studies, and of at least one more feeding study (usually 48 day broiler chicken). This seems to indicate that if such studies were *not* available, she would support the request for whole feed studies, and it is anyhow in contradiction with her reply on question 112, where she dismisses *all* chronic or other studies apart from one acute toxicity study in mice.

905. Besides, she does not address the quality and reliability of the information provided in each application, and in particular she does not address the molecular characterisation data, nor does she addresses the end use of the GM crop, where different target animals might need to be tested before the product can be used safely as feed with these animals .

Detailed comments

906. The European Communities will refer the Panel to its detailed comments on the previous question and in section III on general and methodological issues, where, again, there is ample scientific explanation why these studies may be justified.

907. Whole food studies are necessary to complete the assessment of the safety of new feeds or foods for the following reasons:

> The determination of the nutrients-toxicants (substantial equivalence) can not detect all unintended effects (products);
>
> The level of proteins may be increasing significantly ins successive products (See the comparative levels of CRY IA (b) protein level in the Bt MON 810 compared to that found in Bt 176 maize;
>
> As is well known, acute gavage with recombinant proteins and *in vitro* degradation of purified proteins have limited value;
>
> Alternatively, use of well established protocols for tolerance studies of pharmaceutical are available but sometimes difficult to follow;
>
> Whole food studies can and must be used to complement other safety testing approaches.[306]

908. There are still a lot of methodological issues that need to be clarified for the conduct of whole food studies. The duration of tests on unintended effects must be standardised (42 days for poultry and dairy cows; 100-120 days for pigs and steers). It is not possible to administrate the product at doses that are multiple of the expected human exposure. Extended clinical and histo-pathological studies may not possible and not appropriate for such studies.

909. There is the need, in order to achieve a reasonable level of certainty in the safety assessment, to use a combination of methods. As indicated in of the text FAO/WHO on Expert consultation (Geneva 2000), completing the work of the OECD (1993): " Integration of nutritional and toxicological expertise was needed for the evaluation and be encouraged and facilitated".

> Whole food studies must be performed on specific target species: monogastric for grains, herbivores or ruminants for forages; recombinant proteins are more expressed in leaves (x10 compared to grain); rodents could be used but are more resistant to specific toxicants;

[306] See König *et al*., 2004.Food Chem.Tox. 42-1047.

> Numerous results have been published in the scientific refereed literature on nutritional equivalence: as safe as….. no recombinant material found in animal products (milk, eggs, meat…).
>
> Successive approaches of whole food studies have been performed in the US on RR soybean[307], even as late as 2003:

910. "In vitro degradation and acute toxicity tests were performed on CP4EPSPS protein in order to support that RR soybean was safe[308]. But complementary results from whole food studies have been provided from experiments performed on pigs fed whole soybean meal up to slaughter.[309]. Additional toxicological data have been recently published on mice fed soybean meal, on the basis of data on the testicular development[310].

911. This demonstrates clearly that even for a GM crop released already 10 years ago, a sort of "monitoring" based on whole food studies has been performed for the oldest GM plant placed on the market.

912. The US claims (3rd written rebuttal submission summary) that the request for chronic toxicity when acute studies show no effect are not warranted in the case of NK 603 (page 8). However, just to take one single example, in the case of maize NK603, there where two proteins were expressed (CP4EPSPS and CP4EPSPS L 214 P) and these acute toxicity tests were notably insufficient (See Q. 54).

913. Furthermore, it is clear that different parts of the GM crop may have different properties, and express the individual genes integrated in the genetic modification at different levels. This warrants studies on different target animals, with different diets as regards the plant parts, in order to assess the unintended effects in the different part consumed.

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[307] Safety tested by acute gavage with purified CP4EPSPS and by semi chronic studies on rats and chickens, catfish and dairy cows in B.G. Hammond *et al*., 1996 J. Nutr. 126:717; Safety tested again in pigs (Performance, meat quality) G.L. Cromwell *et al.*, 2002. J. Anim. Sci. 80:708; Absence of protein in muscle of pigs J.C. Jennings *et al.*, 2003.J. Anim. Sci. 81:1447; Safety tested on mouse in long term studies (Foetal, postnatal and testicular development D. Brake *et al*., 2004. Food.Chem. Tox. 42:29.

[308] Harrisson et al., 1996. J. Nutr. 126:728

[309] Cromwell et al., 2002. J. Anim. Sci. 80:708

[310] Brake and Everson, 2004. Food Chem. Tox. 42:29.