

## ANNEX H

### REPLIES BY THE SCIENTIFIC EXPERTS ADVISING THE PANEL TO QUESTIONS POSED BY THE PANEL

#### GENERAL COMMENTS OF THE EXPERTS

##### Dr. Snow

A. WHICH ENVIRONMENTAL CONCERNS ABOUT GM CROPS ARE REALLY "SCIENCE-BASED"?

1. A great deal has been written on this topic, and I will not try to summarize all of it here. Most hypothetical concerns pertaining to environmental effects of GM crops are included in the following list from the Ecological Society of America (Snow et al. 2005). Risk assessments are needed to minimize the likelihood of:

- creating new or more vigorous pests and pathogens;
- exacerbating the effects of existing pests through hybridization with related transgenic organisms;
- harming non-target species, such as soil organisms, non-pest insects, birds, and other flora and fauna;
- disrupting biotic communities, including agroecosystems;
- causing irreparable loss or changes in species diversity or genetic diversity within species.

2. Clearly, many GM crops are unlikely to cause any of these potential problems. Furthermore, it is not logical to group all GM crops into a single category and conclude that they are either inherently safe or inherently dangerous (see Question 103 below). It is important to evaluate new GM crops on a case-by-case basis in each country where the crop will be grown, and to do so using appropriate baseline comparisons. In the United States, for example, each new GM variety is compared to its non-GM predecessor in the context of conventional agricultural practices, and in the context of the nation's overall strategy for how its agricultural, environmental, and trade policies are implemented.

3. Some of the environmental concerns that EC Member States have raised in the documents are similar to concerns that routinely are addressed by regulatory agencies of the Complainants. However, there are also major differences in regulatory goals and policies. For example, the conservation of farmland biodiversity is a much bigger issue in Europe than in the USA or Canada (I am not familiar with the situation in Argentina). In some European countries, much of the non-urban landscape consists of farmland, which is the primary habitat for native insects, birds, and other animals. Also, EC Member States' strategies to manage and/or reduce herbicide applications differ from policies of the Complainants. In addition, the EC requires post-release monitoring to check for unanticipated environmental problems, reflecting a more cautious approach to risk assessment. The overall philosophy of EC Directive 2001/18 differs radically from regulations of the Complainants by emphasizing the possibility of cumulative and long-term hazards that could be caused by GM crops, and the need for precautionary decision-making to avoid irreversible harm to the environment. Ultimately, with regard to the Panel's questions about scientific issues, it may be useful to

acknowledge these major differences between the disputing parties in their environmental and public health goals and their strategies for reaching these goals.

4. Some of the delays in EC regulatory decisions could be related to the time needed to develop new legislation that pertains to labeling (e.g., Regulation 1830/2003). Also, there were many requests for new information to be added to notifications when EC Directive 90/220, which had been in effect since 1990, was replaced by Directive 2001/18 in March 2001. The new Directive includes requirements for labeling and traceability, as well as consideration of socioeconomic and ethical issues.

5. Another question that has been raised in Europe is how to ensure that GM crops can "coexist" with non-GM crops, including both conventional and organic crops, such that current and future labeling standards can be met. I will not attempt to address this socioeconomic and legal issue, other than to note that scientific information about gene flow is often relevant to questions about coexistence. In some cases, further research may be needed to fill information gaps.

6. As a scientific advisor to the Panel, I am restricting my comments to over-arching questions about whether various Member States had valid reasons to conclude that additional scientific research was needed to complete their environmental risk assessments in 1998-2003.

7. Below, I list questions about possible environmental problems that could result from cultivating the specific GM crops that are cited in this dispute. These concerns apply mainly to agricultural landscapes rather than other managed or natural habitats for flora and fauna. For each new GM variety, one can ask whether its widespread cultivation is likely to cause any of the following outcomes to a greater degree than corresponding non-GM varieties:

- a. create or exacerbate weed problems
- b. cause direct or indirect harm to non-target species, including:
  - i. beneficial insects and soil organisms that affect crop yields
  - ii. native flora and fauna, including culturally important species
    - e.g., native butterflies, skylarks, wild relatives of crop plants
- c. lead to the use of more herbicides or insecticides, including more toxic ones, potentially harming flora, fauna, and human health.

#### Gene flow and the concept of "genetic pollution"

8. I will focus most of my comments on the environmental effects of gene flow from GM crops. In this context, the dispersal of transgenes by pollen or seeds is not a problem, in and of itself, unless this process has unwanted biological consequences. From this standpoint, the presence of transgenes in non-GM crops, wild relatives, or weeds can be seen as being part of a normal process that occurs with all crop genes. It is well known that crop genes can disperse widely by means of pollen and seeds. Only certain types of new genes, such as those that confer herbicide resistance or cause harm to non-target species, might lead to unwanted biological consequences.

9. The mere fact that GM crops are regulated may cause some people to conclude that *all* transgenes are risky. I do not agree with this opinion, as I discuss in my answer to Question 103. The process of artificially inserting genes into a plant's DNA can have unintended consequences, such as abnormal growth or development, but it is unlikely that 1) these effects will be ecologically significant in commercially-produced transgenic crops, or 2) they would be more risky than the types of side-effects that arise routinely during conventional breeding. In any case, many scientists agree that genetically modified plants should be judged on the basis of their phenotype – their actual characteristics – rather than the process that is used to develop them (e.g., Tiedje et al. 1989, NRC 2000, Snow 2003).

10. Moreover, there is no reason to expect that transgenes could be harmful simply because they are released in a particular crop's center of diversity (i.e., where many of the crop's wild ancestors or original cultivars occur; e.g., Gepts and Papa 2003, Bellon and Berthaud 2004). This topic has received a good deal of publicity, but there is no reason to expect that the effects of gene flow from modern cultivars on the genetic diversity of wild relatives differ between transgenic vs. nontransgenic crops. As stated above, gene flow does not represent an environmental concern *per se*, unless it results in unwanted biological consequences. If there *are* unwanted consequences, the extent to which the transgenes disperse *via* pollen and seeds may be analogous to the "exposure" term in the simple risk assessment equation "risk = exposure x harm", in which these terms are expressed as probabilities.

11. The documents in this dispute indicate that some Member States consider transgenes to be a type of genetic pollution, even though these genes are unlikely to have any negative effects on plants, animals, human health, or the environment. For example, Greece stated concerns about herbicide resistance transgenes from imported oilseed rape that is not intended for cultivation (see Question 66). Here the perceived risk appears to be that the transgenes would be harmful to native *Brassica* species. I do not agree that this would be a problem because 1) the imported seeds would not be cultivated (though some mix-ups with seed sources could occur), and 2) the transgene in question – a gene that confers resistance to the herbicide glufosinate – is unlikely to be harmful to native *Brassica* species in Greece, should they eventually hybridize with crop plants. Even if transgenic seeds of oilseed rape were to leak into the local farming system, perhaps establishing volunteer populations of oilseed rape, it is difficult to imagine how a transgene that confers resistance to glufosinate could be harmful to wild relatives of the crop.

#### B. SCIENTIFIC UNCERTAINTY DURING 1998-2003

12. Biotechnology companies have been evaluating characteristics of GM crops for over two decades. As of 1998, however, the USA, Canada, and Argentina had only two years of experience with commercial-scale cultivation of transgenic Bt and herbicide-tolerant crops. Independent researchers who study environmental effects of GM crops had even less experience, partly because funding for this research was meager given the scope of key questions (e.g., Tiedje et al. 1989, Snow and Moran-Palma 1997, Snow 2002, Snow et al. 2005). Also, it has often been impossible for independent researchers to gain access to GM plants for field studies that precede deregulation (Dalton 2002). Rigorous ecological research typically requires several years and multiple teams of researchers to answer seemingly simple questions such as:

- Will pollen from Bt maize (corn) be harmful to native butterflies?
- Will volunteer plants from herbicide-tolerant oilseed rape become problematic weeds?
- How will farmland wildlife be affected by the cultivation of new GM crops as compared to current crops?

13. Therefore, from 1998-2003, many ecological questions about current and planned GM crops were just beginning to be addressed.

14. When *additional* scientific knowledge is needed to evaluate new GM crops, each nation's regulators and scientific advisory committees are placed in the difficult position of choosing between expediency and greater certainty. It is not always clear where the distinction lies between what regulators "need to know" vs. what is merely "nice to know". Regulators often have to rely on intuition or small-scale, unpublished studies, some of which lack adequate statistical tests (e.g. Marvier 2002). The routine, agronomic field trials that are conducted by biotech companies prior to deregulation are rarely intended to answer ecological-scale questions, nor would they be sufficient. Moreover, regulators and scientific advisory committees may not have the insight to correctly identify all of the potential risks that should be considered for each GM crop that is proposed for deregulation. In recent years, the hotly contested political, socio-economic, and ethical questions that pertain to GM crops have made it even more difficult to find the appropriate balance between expediency and certainty.

15. Scientific research can play an important role in how GM crops are evaluated by confirming or refuting widely held assumptions. Findings that are published in peer-reviewed scientific journals help define and answer key scientific questions that relate to risk assessments. Below, I include a sample of recent publications (1994-2003) that are relevant to this dispute because they illustrate recent discoveries. Several of these papers challenged widely held assumptions about pollen, gene flow, or herbicide-tolerant crops. The context for these papers should become clear in light of my answers to the questions from the Panel. Papers that appeared in four high-profile journals – Nature, Science, Philosophical Transactions of the Royal Society of London, and Proceedings of the US National Academy of Sciences – may have had the biggest impact on European decision-makers' perceptions about environmental effects of GM crops.

Examples of recent scientific findings about pollen, gene flow, and herbicide-tolerant crops:

(Listed in chronological order; see References for complete citations.)

1. Crop genes can spread from oilseed rape to populations of weedy *Brassica rapa*, contrary to previous expectations.  
Jeorgensen and Andersen 1994. **American Journal of Botany**.
2. Transgenes that confer herbicide resistance can spread from oilseed rape to populations of weedy *Brassica rapa* (not surprising based on the paper listed above, although this article received a great deal of attention).  
Mikkelsen et al. 1996. **Nature**.
3. Oilseed rape transgenes that confer herbicide resistance and spread to wild relatives do not harm weedy *Brassica rapa*, even in the absence of herbicide use.  
Snow et al. 1998. **Molecular Ecology**.
4. Transgenic pollen from Bt maize is harmful to monarch butterfly larvae under laboratory conditions.  
Losey et al. 1999. **Nature**.  
In 2001, several follow-up papers in the **Proceedings of the US National Academy of Sciences** (e.g., Sears et al. 2001) concluded that this is not a problem under field conditions;

in contrast, Jesse and Obrycki (2000; **Oecologia**) *did* find evidence for harmful effects on monarch butterfly larvae under field conditions.

5. Pollen flow among fields of herbicide-tolerant oilseed rape in Canada can lead to the emergence of weedy volunteer plants (from unharvested seeds) that are resistant to three types of herbicides.  
Hall et al. 2000. **Weed Science**.
6. A modeling study showed that more effective control of weeds, for example in fields of herbicide-tolerant crops, could reduce the food supply of declining populations of skylarks in the UK.  
Watkinson et al. 2000. **Science**.
7. Feral populations of oilseed rape can persist outside of cultivated fields for more than eight years, contrary to previous expectations. This study showed that persistent feral populations can originate from earlier varieties of the crop that are no longer planted.  
Pessel et al. 2001. **Theoretical and Applied Genetics**.
8. Transgenes were discovered in locally produced landraces of maize in remote areas of Mexico, despite a national moratorium on planting transgenic maize.  
Quist and Chapela. 2001. **Nature**.  
(This finding was confirmed by further studies, while other conclusions in this controversial paper have largely been discounted.)
9. Unharvested and spilled oilseed rape seeds can acquire secondary seed dormancy and may persist for five years or longer in no-till as well as tilled systems, contrary to previous expectations. Previous studies suggested that seeds from the crop would be unlikely to acquire secondary seed dormancy under no-till conditions.  
Simard et al. 2002. **Weed Technology**.
10. Pollen from oilseed rape fields can travel at least three kilometers away from source fields, thereby allowing cross-pollination to occur over much greater distances than previously expected.  
Reiger et al. 2002. **Science**.
11. Hybridization between transgenic oilseed rape and weedy *Brassica rapa* is inevitable in the UK, further demonstrating difficulties of confining gene flow if this is deemed necessary.  
Wilkinson et al. 2003. **Science**.
12. Widespread adoption of glufosinate-tolerant oilseed rape and sugar beet might be harmful to the biodiversity of agroecosystems in the UK, as compared to effects of currently used non-tolerant varieties.  
Results of government-sponsored Farm Scale Evaluations. **Philosophical Transactions of the Royal Society of London** (several papers, including Firbank 2003, Squire et al. 2003).
13. In Canada, non-GM oilseed rape seeds that were sold to farmers were often contaminated with small amounts of seeds that had transgenic resistance to two herbicides, glyphosate and glufosinate. This exacerbated the emerging problem of volunteer canola plants with glyphosate resistance, a trend that might jeopardize the use of low-tillage weed control.  
Freisen et al. 2003. **Agronomy Journal**.

16. These publications illustrate that even within the specialized area of gene flow and herbicide-tolerant crops, new findings and new unanswered questions repeatedly appeared in prominent scientific journals between 1998 and 2003. This undoubtedly contributed to science-based considerations of various Competent Authorities in Europe. Unfortunately, results from these papers have often been distorted by the media and others, leading to a great deal of flawed information about the actual content and the inherent strengths and limitations of these studies. In this climate of controversy and distrust, some of the publicity about risks of biotech crops has led to exaggerated concerns about environmental risks. At the same time, the biotech industry sometimes is perceived as being unaware of or unconcerned about valid environmental risks that are taken seriously by professional scientists and others (e.g., Gray 2004).

17. As new publications appeared in the scientific literature during 1998-2003, decision-makers saw that transgenes are expected to spread very widely by means of pollen and seeds, and that therefore these genes could show up in certified seeds sold to farmers, farmer-saved seeds, volunteer plants, and weedy or wild relatives of the crop. Even in maize, which has no wild relatives or feral populations in the USA, and which must be purchased anew each year to achieve high yields, experience with Starlink corn showed how difficult it is to prevent unwanted genes from re-appearing year after year. After Starlink was taken off the market, its unauthorized Bt gene (*cry9C*) continued to be present in trace amounts in the US grain supply three years later (USDA 2003, Marvier and Van Acker 2005). Evidence that transgenes could be present in freely reproducing plants over very large areas shows that "calling them back" will not be practical for crops like oilseed rape. Therefore, scientific advisors of some Member States cautioned against approving certain notifications until the environmental and economic effects of commercialization were understood more thoroughly.

18. While new questions were being raised about specific GM crops during 1998-2003, many reports stated that the GM crops grown in North America were neutral or beneficial from an environmental standpoint. Regulatory agencies and biotech companies in the USA assume that transgenic crops that are approved for commercial production are no more risky than nontransgenic crops. The US National Research Council, which is independent of the government, published two critiques of regulatory approvals of the first GM crop varieties in the US (NRC 2000, 2002). These critiques included suggestions for how to improve the regulatory process to make it more rigorous, but they did not question the safety of products that had been approved for marketing. To date, no clear environmental problems have been identified from the cultivation of GM soybean, cotton, or maize in the USA. In Canada, one problem that has arisen from growing herbicide-resistant oilseed rape (canola) is the emergence herbicide-resistant volunteers that are more difficult to manage than normal volunteers (e.g., Hall et al. 2000, Friesen et al. 2003).

19. Here, it is important to recall that the period during which GM crops have been grown commercially is relatively short, having begun in 1996-1997. This is not much time for noticing major environmental effects of GM varieties. In the USA, for instance, little attention has been paid to documenting positive or negative effects on biodiversity empirically, and many types of effects are not expected to be obvious to casual observers (e.g., NRC 2002, Snow et al. 2005).

C. SPECIAL CONCERNS ABOUT THE LONG-TERM EFFICACY OF BT CROPS AND THE HERBICIDE GLYPHOSATE

20. The most widely used transgenes in commercial GM crops are various Bt transgenes and those that confer resistance to glyphosate. These transgenes deserve special consideration because of the environmental and health benefits they can sometimes provide, relative to current pesticide practices, and because their effectiveness could be lost if the target pests evolve resistance to the Bt pesticide or the herbicide glyphosate. This is different from other types of possible risks, such as

greater allergenicity, because it deals with the loss of a benefit rather than the creation of a risk. First, I briefly summarize the benefits of Bt crops.

21. Plant-produced Bt proteins can be more effective at controlling insect pests than chemical sprays because they kill insects that feed inside the plant, where sprays cannot reach, and these proteins are produced continuously throughout the season. In crops like cotton, which requires heavy insecticide applications, the use of Bt cotton substitutes for many of these broader-spectrum insecticides. This can be beneficial to farm workers' health, populations of non-target and beneficial insects, and the environment. Similar benefits can be seen with Bt maize, but only in regions where insecticide use is deemed necessary to begin with. The high specificity, low toxicity, and agronomic benefits of Bt proteins make them extremely valuable, especially in comparison to standard practices in chemical-intensive conventional agriculture.

22. One of the risks associated with Bt crops is that target insects could become resistant to these Bt proteins, and therefore a valuable method of insect control would be lost. Because of these concerns, many countries in which Bt crops are grown have developed formal or informal management strategies to delay the evolution of target insect pests that are resistant to Bt proteins.

23. A somewhat similar situation exists with the herbicide glyphosate (sold as Round-Up<sup>TM</sup>). This herbicide is extremely effective at killing most species of weeds and, compared to many other herbicides, it breaks down relatively quickly after use. Thus, it is much easier and safer to use than herbicides such as 2,4-D, although some crops can thrive with little or no herbicide treatment (baseline levels of herbicide use vary among crops, countries, and farmers). Glyphosate is relatively inexpensive and it is widely available in many countries. In North America, large-scale conventional farmers often use glyphosate for general weed control and for no-till and low-till cultivation, which has several environmental benefits (e.g., reducing erosion and siltation).

24. Despite heavy use of this herbicide over the past twenty years, few weed species have evolved resistance to glyphosate, in contrast to the large numbers of weed species that are resistant to other herbicides like imidazolinone (WSSA 2005). The problem of allowing more and more types of weeds to become resistant to commonly used herbicides is widely recognized by weed scientists, biotech companies, and farmers, but few countries have implemented effective strategies to delay the evolution of resistance.

25. Like Bt, glyphosate has been very popular with farmers and its use has increased substantially with the adoption of glyphosate-tolerant crops. As with Bt, one of the risks associated with the spread of resistance genes in pests (in this case, weeds and volunteer plants) is the loss of a benefit that is associated with this particular herbicide. To summarize, Bt crops and glyphosate offer potential benefits to both farmers and the environment, and scientists are skeptical about whether they could be replaced in kind if target pests – be they insects or weeds – evolve resistance. Therefore, many scientists, farmers, and decision-makers are working on methods to prolong the efficacy of Bt, glyphosate, and glyphosate-tolerant GM crops.<sup>1</sup>

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<sup>1</sup> References Dr. Snow:

*Note: Papers cited in this report are underlined.*

*This reference list includes a large proportion of peer-reviewed papers about gene flow from oilseed rape, which is the focus of my answers. Numerous publications are relevant to these questions, including citations listed in a recent review by Devos et al. (2005).*

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**Dr. Squire**

**Notes on ecological and environmental standards**

26. These notes are intended to summarise my views on some general issues pertaining to the questions set by the panel. The notes touch on –the general weakness of current criteria and standards, particular in relation to ecological topics, by which products of the type under review might be judged, the importance of context and scale when assessing new global products for specific markets, some recent and current research in geneflow and ecological impacts.

**Criteria and standards**

*Information required to prove that a biotech product is safe*

27. No product can be assessed as being absolutely safe. There seems to be general agreement on that. However, there is no general agreement on what should be considered as acceptably safe. As information is first gathered on a product, the likelihood that the product is safe or not may increase quite steeply, but gradually each further piece of information comes to contribute less and less to the likelihood that the product is safe. (The knowledge/safety curve assumes the form of 'diminishing returns'). Among the present arguments, however, there seems no agreement as to when there is

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WSSA – Weed Science Society of America (2005) International survey of herbicide resistant weeds. <http://www.weedscience.org/in.asp> (accessed January 3, 2005).

enough information to satisfy all reasonable parties that a product is safe. This is largely because there is no benchmark or set of standards by which ecological safety can be judged.

28. There is an important set of exceptions to the 'diminishing returns' shape of a knowledge/safety curve. If the body of knowledge reveals a new property of the biotech product, or if the external 'environment' – the context – changes, then the knowledge/safety curve could well steepen again because much additional knowledge is then required to ascertain that the product is safe in relation to the new property or context. In some instances, new properties might emerge only after a product is grown over a period of time or over a wide area of land. Several of the arguments and uncertainties in the dispute over geneflow and contamination are of this type: current knowledge seems insufficient to model and predict the extent of a potential problem.

#### *International Standards and Guidelines*

29. The standards and guidelines referred to in the panel's questions are (to this reviewer) important steps towards an agreed set of standards. They differ in their stringency and required detail. Recommendations are relatively well defined and stringent in the *WTO Sanitary and Phytosanitary Measures*, in *ISPM 11*, and in the *Guideline for the Conduct of Food Safety Assessment of foods derived from recombinant-DNA plants*. The latter in particular requires great detail in characterising and describing the genetic material, plant metabolites and other constituents. Many of the 'requests for further information' by the EC or its member states are consistent with (i.e. not beyond) the level of detail indicated in that publication.

30. Less detailed and prescriptive are the 'Proposed draft annex on the assessment of possible allergenicity.....' and the Annex III of the Cartagena protocol on biosafety to the Convention on Biological Diversity'. Both offer less well defined guidelines, largely because the issues (allergenicity, impact on ecosystems) are complex and, as indicated at paragraphs 2 and 7, there are no or few obvious quantitative criteria on which to base assessments. This weakness of criteria makes risk assessment very difficult in relation to these topics.

31. As many others have said, non-biotech crop plants are not subjected to anything like the same degree of testing as biotech plants. If they had been, the ecological standards might now be in place.

#### Criteria and comparators for ecological effects

32. Many of the arguments around the ecological benefits or harm of biotech crops are carried on without reference to objective criteria. *Annex III of the Cartagena Protocol on Biosafety to the Convention of Biological Diversity* indicates only the general type of information that might be considered. Even when discussing specific products in relation to specific ecosystems, neither the biotech companies nor the responding EC bodies refer to objective criteria when stating that a product is safe or not. For instance, an assessing body might require more information on long-term ecological safety without stating what type of information is necessary to ascertain safety.

33. The problems referred to in paragraph 7 arise because we lack a set of properties –physical, chemical, biological, social and economic – which define that a production system is resilient and 'healthy'. As a stop-gap, ecological studies have compared GM products with non-GM isogenic lines when making initial comparisons on a small scale, and then with another agronomic practice when making comparisons on a field scale. Even when a comparator is used (e.g. current practice), it is not yet clear that the comparator is an appropriate standard, one that will sustain crop production. Neither side in the argument over any product has indicated, even in general terms, what a suitable set of ecological standards might be.

#### Scale and context in assessing biodiversity and ecological impact

34. The arguments in paragraphs 7 and 8 are complicated further because ecological criteria will be influenced by the context – the combination of weather, soil, landscape, the agronomy and economics – of which the flora and fauna are an essential part. The companies seem to have been unaware of, or not appreciated, the context in which biodiversity exists when presenting many of the cases in support of their products. Biodiversity is important primarily because it keeps an ecosystem intact and functioning and makes it resilient to external perturbations. The diversity-function relation – i.e. that diversity in microorganisms, plants and animals is required to maintain essential functions such as decomposition, nutrient transformations, soil formation, detoxification, regulating pests, etc. – has been largely ignored in arguments over the safety of biotech crops (and non-biotech crops).

35. It is necessary therefore that the biodiversity in the cropped field is taken into account as much as the biodiversity of the semi-natural habitats surrounding agriculture. Moreover

- what is considered the valuable biodiversity in one part of the world might be very different from what is considered valuable in another part, and
- a product that is considered to have negligible impact on total biodiversity in one part of the world might have a large effect in another (and vice versa).

36. There are some large differences between Europe and North America in these respects. In many parts of Europe, valuable biodiversity is considered not just that of natural and semi-natural areas (mountains, extensive forests), but must include the flora and fauna within arable fields, both for the reasons in paragraph 9 above (i.e. the diversity-function relation) and because biodiversity in the form of farmland plants, insects and birds is perceived by people as being valuable.

37. *Emergent properties at the landscape scale.* Moreover, it is scientifically unreasonable to assume that an effect (positive or negative), or the absence of effect, of a biotech product when measured at the scale of the single plant or small field plot will be the same when measured at the scale of the field, farm or landscape. Up-scaling of biological effect to the landscape was a major topic of research in the 1990s and still is. Questions include -

- Will cross-pollination frequencies at a field or feral population rise substantially as the proportional area of a donor type increases in the landscape?
- Will volunteer and feral populations become more competitive and longer persisting as they evolve through selection and the incorporation of new traits?
- To what extent will variation in local physical conditions and occasional human error seriously reduce the efficacy of mitigation protocols?

38. One of the main difficulties in predicting the effect of any type of innovation is still the uncertainty over up-scaling. What will happen as more and more of the land area is affected by the innovation? Again, progress is limited by the absence of hard criteria that define resilient or sustainable states at the scales in question.

#### New research since 1998 on relevant ecological topics

39. In part to address issues in paragraphs 8 to 12, a large body of research has been commissioned on ecological impacts and gene flow in Europe. Much of the previous research on GM

plants in the early and mid-1990s compared genetic lines with and without a GM trait, either in containment, or in small areas of field. However, it was increasingly accepted during the 1990s that this was insufficient to provide knowledge of the spread, persistence and ecological effects of biotech crops at scales of the field and landscape. An important watershed was the symposium held in 1999 – *Gene flow in agriculture: relevance for transgenic crops* – which brought together researchers from Europe and North America to compare latest studies on gene flow and persistence of GM and non-GM crops in the environment. This symposium drew attention to the fact that gene flow at the landscape-scale had spatial and temporal features that were not evident at smaller scales of study.

Gene flow in Agriculture: relevance for transgenic crops. (1999) Proceedings of a Symposium held at the University of Keele, 12-14 April 1999. Chaired by PJW Lutman. British Crop Protection Council, Farnham, UK.

40. Both the EC and several EC countries have commissioned multi-partner studies on gene flow and GM impacts. Results are reported for some studies; others are in progress or partly completed. They include the following projects.

- SIGMEA – Sustainable Introduction of GM crops into European Agriculture (<http://sigmea.dyndns.org/>) a EC-funded project which began in 2004 and has the main aims of (a) collating European information on the spread, persistence and ecological effect of the main crop species under review here and (b) developing models and decision support tools for the introduction of GM crops and their coexistence with other forms of cropping. SIGMEA has contributions from 45 partner organisations, and will be conducted over three years. Progress to date indicates that a very large part of the scientific knowledge on this topic is not yet in the public domain, because it is either recently completed and not published or is still in progress.
- ECOGEN – Soil ecological and economic evaluation of genetically modified crops (<http://ecogen.dk>), an EC-funded project in progress, examining the effect of Bt maize and other biotech crops on soil communities and processes at field sites in Denmark and France.
- The Farm Scale Evaluations of GMHT crops compared GMHT and conventional crop management on arable plants and invertebrates at more than 250 sites in the UK. The first findings for spring-sown crops were published in 2003 and the effects of winter oilseed rape on biodiversity will be in 2004. Studies on gene movement and persistence of the GMHT trait are in progress.

The Farm Scale Evaluations of spring-sown genetically modified crops. 2003. Philosophical Transactions of the Royal Society, Biological Sciences, 358, Theme Issue.

41. Botanical and rotational implications of genetically modified herbicide tolerance in winter oilseed rape and sugar beet (BRIGHT project). Several years of study at 5 sites in the UK comparing the management of various herbicide tolerant varieties.

Final report at [www.hgca.com](http://www.hgca.com) and look under *Crop research*.

42. GMO Guidelines Project, under the auspices of the International Organisation for Biological Control (IOBC), funded by the Swiss Development Cooperation; the aim is to develop international

biosafety testing guidelines for transgenic plants, with emphasis on ecological communities and processes in developing countries, but in principle applicable to Europe.

Hillbeck A. and Andow DA (Eds) 2004. Environmental risk assessment of genetically modified organisms. Volume 1. A case study of Bt maize in Kenya. Eds. CAB International, Wallingford, UK. Further case studies are in progress.

43. Additionally, there are many other country-specific studies, notably in Germany, France, Denmark and the UK that are still in progress.

44. In summary, the understanding of environmental and ecological risk and benefit has lagged behind the understanding of most other forms of risk. There is no set of agreed criteria for defining 'ideal' arable ecosystems and this has restricted logical and rational argument on ecological impacts in most of the cases under review. Research is in progress in a range of European contexts to provide the scientific information which could be used to define the necessary criteria.

#### Annex to Notes. Persistence and movement of genes among crops, weeds and wild relatives

45. The summary in Table 1 is offered as background to several of the panel's questions that refer to persistence and spread of GM plants. Several of the crops under review have the potential to shed seed and leave descendents. The following definitions are used. Volunteer(s): plants that originate from seed or vegetative material shed or left by a crop and that inhabit fields, usually emerging as a weed within a crop. Feral: plants that originate from seed or vegetative material shed or left by a crop and that exist outside fields, in waysides and the margins of agriculture. (Some authors use the term feral for plants descended from a crop whether they are found inside or outside fields.) The table shows that transmission among crops, feral/volunteer plants and wild relatives is highly species-specific. Persistence and spread are generally greatest for oilseed rape, low for beet providing the crop is prevented from flowering, very low for maize except between flowering maize crops and negligible for cotton as presently grown in EC countries.

46. A relevant factor in all arguments over biotech oilseed rape is that the perception of oilseed rape as a weed pest in Europe has increased as more findings from research became available from the mid-1990s and through the early 2000s. Notable facts include –

- It persists over 5 to 10 years at relatively high density in the soil (e.g. around 100 seeds per square metre).
- It is very widespread in the arable seedbank, commonly among the top 10 species within fields and occurs frequently as a wayside plant.
- Its re-seeds in subsequent crops of oilseed rape.
- Pollen-transfer causing low-frequency cross-pollination occurs over several kilometres: insect vectors are important in moving pollen.

47. For the sake of balance, oilseed rape should also be seen as a valuable and flexible 'break crop' in cereal rotations; it needs lower agrochemical inputs than many other crops and supports a high level of in-field biodiversity.

Table 1. The ability of the crops to disperse genetic material through feral or volunteer progeny, pollen movement and outcrossing to form hybrids in Europe. Author's summary.

	Beet	Maize	Oilseed rape	Cotton
Feral / volunteer weeds persistence in field	medium	zero <sup>1</sup>	high	zero
persistence outside field	medium	low	medium	zero
Pollen transfer crop to crop	low <sup>2</sup>	high	high	low
crop to feral /volunteer	low <sup>2</sup>	zero	high	zero
crop to wild relative	low <sup>2</sup>	zero	low	zero
Crossing frequency (on arrival of pollen at flowering plants)				
crop to crop	high	high	high	low
crop to feral	high	zero <sup>1</sup>	high	-
crop to wild relative	high (local)	zero	low	-

<sup>1</sup> In most of Europe; potential for some survival in the south.

<sup>2</sup> Provided few or no crop plants allowed to flower.

48. The presence of a GM construct should not markedly affect the qualities in Table 1 nor the status of oilseed rape and other crops as a weed. However, if the GM construct is accompanied by genes that make the plant more or less male-fertile than other sexually compatible plants, then the GM trait might be spread differentially. Oilseed rape can again be used as an example since it varies commonly in male fertility (i.e. the proportion of plants that produce pollen): in some crop varieties all (or nearly all) the plants are male fertile, but other varieties plants can be mixtures of male-fertile (20%) and male sterile (80%) individuals. Also, volunteers (including GM volunteers) produced from seed shed by some types of GMHT crop plant can be variously male fertile or male sterile. Generally, crops and plants that have more male sterility get more cross-pollination from other fields or plants than those that are fully fertile. It is important to know when assessing the risk of spread and persistence whether the variety and its offspring are fully, partly or not male fertile.

**Dr. Snape**

49. The comments below relate to difficulties experienced by the Expert in provided a full exposition in answering questions No. 12, 16, 18 and 25, concerning the molecular characterisation of three exhibits, EC-062, EC-063 and EC-067.

50. All the information on the molecular characterisation of a GMP (genetically modified plant) would greatly benefit from a summarisation and compilation into a single document for each exhibit. Currently, the evaluation of an exhibit requires the mining of information in more than 100 attachments often representing 1000 pages. Moreover, each attachment contains a mixture of information relative to different aspects of the risk assessment (transgene structure, expression, phenotypic stability, toxicity or allergenicity of the product etc. ...). This greatly complicates the assessment and presents a significant risk that not all relevant information will be examined by each expert. This reviewer would recommend that all information relative to an exhibit is organised

according to the structure of the EFSA-Q-2003-005 guidance document Section III "Information required in applications for GM plants and/or derived food and feed". Data initially provided by the applicant as well as the subsequent questions and answers should be kept, by topic, in the appropriate part A, B, C or D of section III. This should allow experts in different fields to have a simplified and exhaustive access to the relevant information.

51. This reviewer has some concerns about the criteria set by in the guidance document EFSA-Q-2003-005 section III-D2 "information on the sequences actually inserted or deleted". The use of the term "insert" can be, and often has been in the exhibits, a source of confusion. In some exhibits "insert" is understood and presented as the DNA sequence to be introduced, in others as the integrated transgenic coding sequence, in others as the integrated expression unit (promoter:coding sequence:polyA signal) and in others as the entire transgenic locus. In the past, this has led applicants to produce Southern blots and PCR-based analyses of variable quality and information content. This has also led to a large number of subsequent requests for additional molecular information by experts. This situation could be avoided if the information was initially requested in term of structural (and functional) analysis of each transgenic locus. A transgenic locus is here defined as a genetic location where transgenic sequences are linked (i.e. co-segregating at meiosis when integrated into the nucleus or remaining physically linked when part of other genetic structures). A transgenic locus can include some plant intervening DNA between transgenic sequences. Molecular analysis in terms of a transgenic locus is particularly relevant for GM plants produced using *Agrobacterium*-based transformation technologies as they often contain transgenic sequences dispersed over several loci into the plant nuclear genome. Southern or PCR analyses of multi-locus GM plants can be informative in terms of total copy numbers but have little relevance regarding the structural organisation of each transgenic locus (copy number, rearrangement level etc...). This Expert is of the opinion that the information required in section III-D2 should be re-organised as follows:

- (1) Sub-cellular location of transgenic sequences (nucleus, chloroplasts, mitochondria or maintained in a non-integrated form) [*same as part of section IIID2d in EFSA-Q-2003-005*]
- (2) When transgenic sequences are integrated into the nucleus, the number of independent transgenic loci. This is primarily obtained from inheritance pattern determined using the structural analysis of transgenic sequence(s) in progenies following appropriate self- or cross- pollination [*similar to part of section IIID2d in EFSA-Q-2003-005*]
- (3) Information about each transgenic locus. When more than one transgenic locus is present in the GM plant, the molecular characterisation of each locus should be conducted independently when possible (i.e. when the GM plant is fertile).
  - (a) The size and copy number of all detectable transgenic sequences, both complete and partial [*same as section IIID2a in EFSA-Q-2003-005*]
  - (b) The organisation of the transgenic sequences at each transgenic locus and the methods used for characterisation [*same as section IIID2b in EFSA-Q-2003-005*]
  - (c) In the case of deletion(s), size and function of the deleted regions(s) [*same as section IIID2c in EFSA-Q-2003-005*]  
"Applicants should demonstrate ..... which molecular analysis is but one" [*same as section IIID2 in EFSA-Q-2003-005*]

- (d) All sequence information including the location of primers used for detection [same as part of section IIID2e in EFSA-Q-2003-005]  
"When *events* have been combined by interbreeding ..... transformation event for risk assessment purposes" [same as section IIID2 in EFSA-Q-2003-005]

52. Please note that inheritance patterns based on the molecular analysis of transgenic sequences in progenies are rarely presented in the exhibits. Generally, the segregation of transgene phenotype (based on transgene expression in the progenies) is used to estimate inheritance pattern. However it is well documented in the literature that the segregation of transgene phenotype is not always a reliable indicator of either transgene inheritance or transgene linkage as only expressing (i.e non-silenced) transgene copies are detected by expression studies. This is particularly relevant when monitoring the inheritance of non-functional transgenic sequences or when more than one copy of the transgene(s) has been introduced into the plant genome. When a single transgene copy is integrated into the plant nucleus, segregation studies based on transgene expression are satisfactory to estimate inheritance patterns.

53. Please also note that the determination of genomic flanking sequences is not enough in itself to ensure that no additional unwanted DNA sequences (such as antibiotic resistance genes from vector backbone) are present at a given transgenic locus or overall in the genome.

54. This Expert has some concerns about the criteria set by in the guidance document EFSA-Q-2003-005 in sections III-D5 "Genetic stability of the insert and phenotypic stability of the GM plant". The current requirements are to "demonstrate the inheritance pattern and stability of the trait(s) introduced". This does not provide avenues to monitor potential recombination or deletion within a transgenic locus across generations. This point reiterates, as expressed in 2 above, the need to focus the molecular analysis at the transgenic locus level. It is documented that large, rearranged and complex transgenic loci such as those generated by direct transfer of DNA (particle gun bombardment etc...) can be prone to such genetic rearrangements across generations. A comparative Southern blot analysis of primary and some transgenic progeny plants using a selection of restriction digests and probes should be adequate to assess the structural stability of each transgenic locus.

55. Overall this Expert feels that the molecular characterisation of the GM plants is probably one of the few areas where applicants have the opportunity to provide non-debatable and definitive information. This information can also be obtained using routine molecular techniques at a reasonable cost. In this context it is surprising that the opportunity of providing a full molecular characterisation of GM plants is not always taken by the applicants. In the case of a small transgenic locus (up to 10,000 nucleotides) this Expert would even suggest that applicants produce a full sequence of the transgenic locus. Most of the information necessary to produce an effective sequence is already required by EFSA-Q-2003-005 (genomic flanking sequences, architecture of the transgenic locus determined by Southern blot analysis). This sequence of the transgenic locus and flanking plant genomic region along with a basic inheritance study at the structural level (using dot blot or PCR) and a Southern blot analysis of the primary transgenic plant and a few progenies should provide a compelling case for the molecular characterisation of each exhibit. When a single copy of each transgene is present in the GM plant at one locus, this analysis can be simplified by using the segregation of transgene phenotype to estimate the inheritance and stability of the transgenic locus. This reviewer understands that large and more complex transgenic loci, such as those produced by particle gun bombardment, may not be easy to sequence. In this later case, particular attention should be given to the molecular stability of the transgenic locus structure over generation (see comment 3 above).

56. In conclusion I suggest:
1. The requests from the EC for information from the companies could have been more precise in its requirements, points 2 and 3 above.
  2. The information collected by the EC could have been compiled more systematically, point 1 above.
  3. Companies could have provided a full molecular characterization of the GM plants in the initial submissions, point 4 above.

## **QUESTIONS TO EXPERTS**

### **General Questions**

**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?**

### **Dr. Nutti**

57. According to FAO/WHO (2000), DNA transfer from plants to microbial or mammal cells, under normal circumstances of dietary exposure, would require all the following events to occur:

- (a) the relevant gene(s) would have to be released, probably as a linear fragment;
- (b) the gene(s) would have to survive nucleases in the plant and in the gastrointestinal tract;
- (c) the gene(s) would have to compete for uptake with dietary DNA;
- (d) 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
- (e) the gene(s) would have to be inserted in the host DNA by rare repair or recombinant events.

58. 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. 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.

59. The transfer of marker genes which confer resistance to kanamycin, ampicillin and streptomycin to bacteria in the human gut is unlikely to present a significant health impact since bacteria resistant to these antibiotics are already spread all over or are naturally found in the human gastrointestinal tract (Smalla et al., 1993; Calva et al., 1996; Shaw et al., 1993; Smalla et al., 1997). Besides, kanamycin/neomycin and streptomycin are rarely used for humans due to their collateral effects (WHO 1993).<sup>2</sup>

**Question 2: 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?**

- (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) **Are these consequences relevant to the specific types of ARMG currently used in the products at issue in this dispute?**
- (c) **If such risks have been identified, what risk management options are available to mitigate those risks and what is their efficacy?**

**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**

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<sup>2</sup> References: FAO/WHO. Safety aspects of genetically modified foods of plant origin. Report of a Joint FAO/WHO Expert Consultation on Food Derived from Biotechnology, Geneva, Switzerland, 29 May-2 June 2000. Rome: Food and Agriculture Organization of the United Nations. 2000. [www.fao.org/es/esn/gm/biotec-e.htm](http://www.fao.org/es/esn/gm/biotec-e.htm)

CALVA, J.J.; SIFUENTES-OSBORNIO, J.; CERON, C. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. *Antimicrob. Agents Chemother.*, vol. 40, pp. 1699-1701, 1996.

SHAW, K.J., RATHER, P.N., HARE, R.S. AND MILLER, G.H. Molecular genetics of aminoglycoside resistances genes and familial relationships of the aminoglycosidemodifying enzymes. *Microbiol. Rev.*, vol. 57, pp. 138-163, 1993.

SMALLA, K.; VAN OVERBEEK, L.S.; PUKALL, R.; VAN ELSAS, J.D. Prevalence of nptII and Tn5 in kanamycin resistant bacteria from different environments. *FEMS Microbiol. Ecol.*, vol. 13, pp. 47-58, 1993.

SMALLA, K.; WELLINGTON, E.; VAN ELSAS, J.D. Natural background of bacterial antibiotic resistance genes in the environment. *Nordic Seminar on Antibiotic Resistance Marker Genes and Transgenic Plants*, p 8-16. June 12-13, 1997, Oslo, Norway. The Norwegian Biotechnology Advisory Board.

Workshop. World Health Organisation, Geneva.

**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?**

**Dr. Andow**

Scientific evidence:

60. In the answer to this question, I will concentrate on Bt maize and Bt cotton. Yes, there is some scientific evidence to support the hypothesis that wide-spread cultivation of Bt crops adversely affects non-target organisms which may be exposed to such crops under typical agricultural practice. However, this evidence is insufficient to establish the hypothesis that such adverse effects are expected to occur.

61. The review of non-target effects of Bt plants in EC-149 covered 13 laboratory studies evaluating potential hazard, and 14 field studies aimed at evaluating potential risk. Of the 13 lab studies reviewed, I conclude that five studies did not expose the test organisms properly and therefore are irrelevant hazard evaluations (an organism must be exposed to evaluate hazard). In two additional studies, four of eight test species were not properly exposed. Of the remaining, seven species were studied.<sup>3</sup> Of these seven, six were on Bt maize and none were on Bt cotton. Bt toxin had significant adverse effects on two of these six species, the collembolan *Folsomia candida* (a soil organism) and the lacewing *Chrysoperla carnea*. In neither case would adverse effects on these species been predicted based on the known spectrum of toxicity of the Cry toxin. There have been no studies to follow up the result with *F. candida*. Hence, Dr. Andow concludes that **there is a possible hazard (adverse effect) to collembola** from Cry1Ab Bt maize, but until this is confirmed, he cannot conclude that there is a potential risk to collembola. There have been numerous studies that confirm that *C. carnea* is somehow adversely affected by Cry1Ab toxin.<sup>4</sup> Hence, he concludes that **there is a potential risk to *C. carnea*** from Cry1Ab Bt maize.

62. Of the 14 field studies reviewed in EC-149, six used plot sizes larger than 0.1 ha.<sup>5</sup> Because most of the study organisms are mobile at spatial scales much larger than the plot sizes used, it is necessary to have larger plot sizes and to tailor the sampling methods to detect possible transient differences in population sizes between treatments. Otherwise, false negatives become problematic. The observed lack of significant differences between Bt and non-Bt treatments in nearly all the studies with small plot sizes is difficult to interpret, because the absence of an observed difference in these kinds of experiments is not a good indication that there were no differences. The only differences

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<sup>3</sup> *Folsomia candida*, *Coleomegilla maculata*, honey bee adults, *Hippodamia convergens* adults, 2 parasitoid species, *Chrysoperla carnea*.

<sup>4</sup> Hilbeck, A., Baumgartner, M., Fried, P.M. & Bigler, F. (1998a). Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environ. Entomol.*, 27, 480-487. Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A. & Bigler, F. (1998b). Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea*. *Environ. Entomol.*, 27, 1255-1263. Hilbeck, A., Moar, W.J., Pusztai-Carey, M., Filippini, A. & Bigler, F. (1999). Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea*. *Entomol. Exp. Appl.*, 91, 305-316. Dutton, A. Klein, H., Romeis, J. & Bigler, F. (2002). Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecol. Entomol.* 27, 441-447. Dutton, A. Klein, H., Romeis, J. & Bigler, F. (2003) Prey-mediated effects of *Bacillus thuringiensis* spray on the predator *Chrysoperla carnea*. *Biol. Contr.* 26, 209-215.

<sup>5</sup> Lozzia and Rigamonti 1998a. *ATTI Giornate Fitopatologiche*, 223-228. Lozzia et al. 1998. *Notiziario sulla Protezione delle piante* 8: 27-39. Lozzia and Rigamonti 1998b. *IOBC Bulletin* 21: 171-180. Lozzia 1999. *Boll. Zool. Agr. Bachic. Ser. II* 31: 37-58. Orr and Landis. 1997. *J. Econ. Entomol.* 90: 905-909. Hardee and Bryan 1997. *J. Econ. Entomol.* 90: 663-668.

detected in these small plot studies were in the potato experiment,<sup>6</sup> which will not be discussed here. Of the remaining six, two studies had insufficient replication.<sup>7</sup> This low level of replication might allow the researchers to detect only differences larger than an order of magnitude. This detection threshold is much larger than what would be considered an adverse effect, so it is possible that some adverse effects were not detected. The remaining four studies had an equivalent of eight replications in each of two years. No adverse effects were observed. Unfortunately, the sampling methods were not sufficiently tailored to detect differences in population size. Taken as a whole, excepting the results on Bt potato, the other 13 studies would indicate that very large, order of magnitude adverse effects on non-target species are unlikely. However, the data do not address the likelihood of other large adverse effects on non-target organisms. Additional research would be needed before claims about safety can be supported with scientific evidence. In particular, the **cited field studies do not enable an evaluation of the likelihood of risk to *C. carnea*.**

63. EC-150 is a scientific paper that evaluated the effects of Bt maize litter (Bt11) on the earthworm *Lumbricus terrestris* in laboratory and semi-field experiments.<sup>8</sup> There were no effects on survival or growth of immature earthworms in the semi-field experiment. There were no difference in adult survival or growth during the first 160 days in the laboratory experiment. Adults were about 20% smaller with Bt litter than non-Bt litter at 200 days. In total and compared with other published results, the results of this study indicate that adverse effects on *L. terrestris*, if they exist, are likely to be subtle. In addition, they suggest that **there is a possible hazard to earthworms** from Cry1Ab Bt maize litter, but until this is confirmed, the Expert cannot conclude that there is a potential risk to earthworms.

64. EC-151 documents a novel route of exposure of soil organisms to Cry1Ab toxin from Bt maize.<sup>9</sup> Cry1Ab toxin is exuded from living maize roots. There has been some controversy as to whether the exudates are from damaged root cells or from a process involving living root cells, however, the evidence suggests that there is a process involving living root cells. This result was not anticipated. Theoretically, the Cry1Ab protein was considered too large to be exuded from living plant roots. **This study does not document a possible adverse effect of Bt maize**, however, it demonstrates that species inhabiting the maize root rhizosphere can be exposed to Cry toxin. This is significant because these species had been considered not at risk previously, and opens the possibility that unanticipated adverse effects to rhizosphere species might be identified.

65. EC-152 demonstrates that Cry toxins persist, accumulate and remain insecticidal in soil by binding to humic acids in soils.<sup>10</sup> Previous work had demonstrated similar results in binding to clay in soils.<sup>11</sup> Moreover, the toxins maintain toxicity for at least 234 days (the longest time examined).<sup>12</sup> Together these studies demonstrate that Cry toxins will persist in soils much longer than previously believed, and that the mechanism of persistence is related to adsorption to clay particles and humic acids in the soil. Together with EC-151, these studies demonstrate that soil organisms are likely to be exposed to Cry toxin via root exudates and litter. **None of these studies document a possible**

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<sup>6</sup> Riddick et al. 1998. *Annals Entomol. Soc. Amer.* 91: 647-653.

<sup>7</sup> Orr and Landis. 1997. *J. Econ. Entomol.* 90: 905-909 had 3 replications and conducted the experiment in only one year. Hardee and Bryan 1997. *J. Econ. Entomol.* 90: 663-668 had 2 replications in each of two years.

<sup>8</sup> Zwahlen et al. 2003. *Molecular Ecology* 12: 1077-1086.

<sup>9</sup> Saxena et al. 1999. *Nature* 402: 480.

<sup>10</sup> Crecchio and Stotzky 1998. *Soil Biol. Biochem.* 30: 463-470.

<sup>11</sup> Venkateswerlu and Stotzky 1992. *Current Microbiology* 25: 1-9. Tapp et al. 1994. *Soil Biol. Biochem.* 26: 663-679. Tapp and Stotzky 1995a. *Appl. Environ. Microbiol.* 61: 602-609. Tapp and Stotzky 1995b. *Appl. Environ. Microbiol.* 61: 1786-1790.

<sup>12</sup> Tapp and Stotzky 1998. *Soil Biol. Biochem.* 30: 471-476.

**adverse effect of Bt maize.** Together, however, they suggest that more species in the soil may be at risk than previously expected. These results suggest that additional studies may be needed to evaluate these possibilities.

66. During 1999, concerns were expressed that monarch butterflies would be adversely affected by Bt maize pollen falling on their host plant, common milkweed.<sup>13</sup> This result was followed up with a series of studies to assess the risk to monarchs.<sup>14</sup> The authors of these follow-up studies concluded that the risk to monarch populations was insignificant for Mon810 and Bt11, because only a small proportion of butterflies would be exposed to these and the toxicity of pollen was low.<sup>15</sup> The events and findings associated with these studies are summarized in an excellent review.<sup>16</sup> Recent studies,<sup>17</sup> however, have revealed a higher toxicity of Bt pollen and anthers than found in previous studies. Although some suggested that the risk to monarchs remains insignificant,<sup>18</sup> a close reanalysis of the issues may allow other interpretations of risk to monarchs.

67. Most these same papers also addressed the potential risk to monarchs from Event 176 BT maize. Although they concluded that there was no risk to monarchs from Event 176,<sup>19</sup> this was based largely on the assumption that Event 176 would not be used very much in production. Had Event 176 gained greater market share, it would likely have put monarchs at risk.<sup>20</sup>

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<sup>13</sup> Losey et al. 1999. *Nature* 399: 214.

<sup>14</sup> Hellmich, R.L., Sigfried, B.D., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R., Spencer, T., Bidine, K.D., Daniels, M.J. & Lewis, L.C. (2001). Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. *PNAS*, 98, 11925-11930. Oberhauser, K.S., Prypsby, M.D., Mattila, H.R., Stanley-Horn, D.E., Sears, M.K., Dively, G., Olson, E., Pleasants, J.M., Lam, W.-K.F. & Hellmich, R.L. (2001). Temporal and spatial overlap between monarch larvae and pollen. *PNAS*, 98, 11913-11918. Pleasants, J.M., Hellmich, R.L., Dively, G.P., Sears, M.K., Stanley-Horn, D.E., Mattila, H.R., Foster, J.E., Clark, P. & Jones, G.D. (2001). Corn pollen deposition on milkweeds in and near cornfields. *PNAS*, 98, 11919-11924. Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried B.D. & Dively, G.P. (2001). Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment. *PNAS*, 98, 11937-11942. Stanley-Horn, D.E., Dively, G.P., Hellmich, R.L., Mattila, H.R., Sears, M.K., Rose, R., Jesse, L.C.H., Losey, J.E., Obrycki, J.J. & Lewis, L. (2001). Assessing the impact of Cry1Ab-expressing corn pollen on monarch larvae in field studies. *PNAS*, 98, 11931-11936.

<sup>15</sup> Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried B.D. & Dively, G.P. (2001). Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment. *PNAS*, 98, 11937-11942.

<sup>16</sup> Oberhauser, K.S. & Rivers, E.R.L. (2003). Monarch butterfly (*Danaus plexippus*) larvae and *Bt* maize pollen: a review of ecological risk assessment for a non-target species. *AgBiotechNet*, 5, 1-7.

<sup>17</sup> Anderson, P.L., Hellmich, R.L., Sears, M.K., Sumerford, D.V. & Lewis, L.C. (2004). Effects of Cry1Ab-expressing corn anthers on monarch butterfly larvae. *Environ. Entomol.*, 33, 1109-1115. Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M. & Anderson, P.L. (2004). Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environ. Entomol.*, 33, 1116-1125. Jesse, L.C.H. & Obrycki, J.J. (2004). Survival of experimental cohorts of monarch larvae following exposure to transgenic *Bt* corn pollen and anthers. In: *The monarch butterfly. biology and conservation* (eds. Oberhauser, K.S., Solensky, M.J.). Cornell University Press, Ithaca, pp. 69-75.

<sup>18</sup> Dively, G.P., Rose, R., Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Calvin, D.D., Russo, J.M. & Anderson, P.L. (2004). Effects on monarch butterfly larvae (Lepidoptera: Danaidae) after continuous exposure to Cry1Ab-expressing corn during anthesis. *Environ. Entomol.*, 33, 1116-1125.

<sup>19</sup> Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Oberhauser, K.S., Pleasants, J.M., Mattila, H.R., Siegfried B.D. & Dively, G.P. (2001). Impact of *Bt* corn pollen on monarch butterfly populations: a risk assessment. *PNAS*, 98, 11937-11942.

<sup>20</sup> NRC (National Research Council). (2002). *Environmental effects of transgenic plants: the scope and adequacy of regulation*. National Academies Press, Washington, DC, USA.

68. As mentioned in paragraph 61, *C. carnea* is under a potential risk from Bt maize. Although this has not yet been studied in the field, Dr. Andow expects that the actual risk to *C. carnea* would not be large. In maize in temperate regions, this species feeds primarily on aphids, mites, thrips, and lepidopteran eggs and larvae. Previous studies have shown that the main aphid on maize does not contain any Cry toxin when feeding on Bt maize, and that mites contain the Cry toxin, but they do not adversely affect *C. carnea*. It is not known if thrips or Lepidopteran eggs contain Cry toxin. Adverse effects on *C. carnea* have been documented only when it feeds on diets containing Cry toxin or on Lepidopteran larvae that had fed on Bt maize. Any adverse effect from Lepidopteran larvae and possibly thrips and eggs would be diluted by feeding on aphids and mites. Consequently, he believes that any adverse effect on *C. carnea* in the field will be subtle and difficult to detect in the field.

69. Relatively few non-target studies have been conducted on Bt cotton. This limits discussion of potential risks.

70. It has been argued that the experience in using Bt maize and Bt cotton in the US provides evidence that there are no adverse effects on non-target organisms. It is true that Bt maize and Bt cotton have been used widely in the US, and it is also true that no adverse effects to non-target species have been reported and confirmed. However, these two facts do not imply that there have been no adverse effects on non-target organisms, because there is virtually no monitoring for such adverse effects. If such effects are not looked for, it is unlikely that they will be found, even if they exist. It can be reliably argued that the US experience with these crops suggests that there have been no immediate catastrophic adverse effects to non-target species. Such catastrophes would have become noticeable even if they were not actively looked for. However, it is more likely that adverse effects, if they exist, will be more subtle, and would not be readily noticed. Hence, **the US experience does not imply that there are no adverse effects on non-target species.**

71. In any event, Cry toxins are unlikely to adversely affect mammals through direct toxicity. They exert their toxic effect by binding first with specific glycolipids in the gut. These protein-glycolipid complexes then bind to specific receptors (cadherin-like receptors) on the wall of the gut. The entire family of the specific glycolipid and receptor molecules are absent from mammals, thus Cry toxins cannot act in their normal way in mammals.

72. Conclusion. Possible hazards have been identified and potential risks are evident, however, it cannot be concluded that an actual non-target risk of Bt maize or Bt cotton has been fully characterized. However, it is likely that if the analysis is completed, that there will be a documented risk of **some Bt maize events to monarch butterflies.**

#### Comparison with Bt insecticides:

73. There are significant differences in exposure probability and exposure routes. Bt insecticides have low likelihood of a non-target risk because they do not persist very long after application. The Cry toxin is degraded rapidly under UV light, and efficacy of the insecticide is lost usually within a few days. This contrasts with Bt crops, which produce Cry toxin constitutively over the entire growing season at high concentrations throughout the plant tissue, and as noted above, can persist in the soil.

74. It is theoretically possible that hazards will be different, but this depends on the protein structure of the Cry toxin in the plant compared with the insecticide. For example, Event 176 Cry1Ab is significantly smaller than Cry1Ab toxin in the bacterium, *Bacillus thuringiensis*. The full protein in the bacterium must be shortened to a form that shows active toxicity. Event 176 protein is already in an active form. Thus, if the shortening process affects hazards, then Event 176 protein may exhibit a

different spectrum of hazards than the bacterium protein. However, it has not been scientifically demonstrated that this difference in structure actually affects hazard. There are also differences between Mon810 and Bt11 Cry1Ab and the Cry1Ab in the bacterium. There appears to be little difference between Event 1507 Cry1F and the Cry1F in the bacterium.

75. Both are unlikely to adversely affect mammals. The Bt insecticides have undergone significant number of studies on mammalian toxicity, and none have been found to be toxic to any of the mammals studied. Coupled with paragraph 71, it is unlikely that either Bt crops or Bt insecticides will adversely effect mammals through direct toxicity.

Risk management options:

76. Risk management measures should be commensurate to the risk.<sup>21</sup>

77. One risk management option is to prohibit the use of the Bt crop. If this option is considered, it should be necessary to provide a worst-case risk assessment to provide a basis for concluding that a prohibition is commensurate to the risk. For example, what would be the harms if all earthworms were 20% smaller after feeding on Bt maize litter for more than 160 days (see paragraph 63)?

78. Another approach would be to limit the area of the transgenic crop until a reasonable assessment of the possible hazards and potential risks are completed. As the information is gathered, the risk management measure can be modified consistent with the principle of "modification."<sup>22</sup>

79. For actual risks, such as the presumed risk to monarchs, geographic restrictions in use of the Bt crop could be considered. Incentives to use alternatives to the Bt crop can be provided. Disincentives for the use of the Bt crop could be imposed. Monitoring of the population at risk could be required, although this would likely be more expensive than restricting or managing use, and may be difficult to justify based on the principle of equivalence.<sup>23</sup>

80. Efforts to improve risk assessment procedures could be viewed as a form of risk management. Improved procedures<sup>24</sup> would be more effective at identifying potential risk, reducing uncertainty and allowing risk assessment to proceed more rapidly.

81. Another option would be to have no risk management. Here it seems necessary to have a risk assessment that concludes that there are no significant non-target risks and that unanticipated effects will be either absent or insignificant.

82. By no means should the Panel assume that this is an exhaustive list of possible management options. These are suggestions of approaches to risk management emphasizing avoiding risk. Approaches that emphasize mitigation of risk or tolerance of risk could also be considered.

**Dr. Squire**

83. Bt crops are grown widely in some regions of the world. They are used to target crop-specific pests, with a consequent, intended benefit that less chemical insecticide is used. They have a potential disadvantage, in that they might harm other organisms in the ecosystem. Among these are the

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<sup>21</sup> ISPM 11, 3.4.

<sup>22</sup> ISPM 11, 3.61.

<sup>23</sup> ISPM 11, 3.4.

<sup>24</sup> For example, Andow, D. A. & Hilbeck, A. (2004). Science-based risk assessment for non-target effects of transgenic crops. *BioScience*, 54, 637-649.

predators and parasitoids that feed on the pests, and organisms not normally reached by chemical insecticides. Important examples of the latter are soil fauna that might be exposed to the toxin if it occurs in or moves out of roots, and detritus feeders and other decomposers that encounter the toxin in dead or dying plant matter. There are therefore arguable advantages and disadvantages in the use of the product. The emerging results from North America show that pesticides have been reduced, but not in all instances – context is important. Much less information on non-target organisms has come from existing Bt maize-growing areas.

84. Potential for ecosystem effects: Assessments of potential ecological harm or benefit in areas such as Europe have so far relied largely on findings from research in the laboratory or in contained systems in which the Bt plant and organisms tested have no, or restricted, contact with the outside world. Three of the exhibits referred to in Q.3 (EC-150, -151 and -152) are among this body of research. The potential for ecological effects is indicated by these and other studies, and some of them have shown that the toxin might well affect soil organisms. Exhibit EC-149 is a review of laboratory and field data up to 1999, and concludes that, because of the small area and short duration of the experiments, only limited inferences can be made about large scale or long term ecological effects. Since then, there have been few field-scale experiments on Bt crops in Europe comparable to those in the present ECOGEN project, from which results should be available in 2005.

85. Scaling, timescales and dilution. The published studies do not demonstrate one way or the other what would happen if Bt maize were grown widely in Europe. There is no general consensus among scientists of what would happen when a Bt crop is introduced into any new region. It is argued by some that there could be a range of unforeseen, negative effects. In support of this view, the authors of Exhibit EC-149 write:

Although such regulatory trials provide valuable initial toxicity information, basing ecological assessments solely on bi-trophic feeding trials that provide the insecticidal protein in a highly processed form directly to the non-target organism is not sufficient. Ecologically important interactions between plants and herbivores and natural enemies/non-target organisms .... may be missed.

86. The implication here is that exposure to the Bt plant over wider spatial zones, and season after season, might cause negative ecological effects that are not observed in short-term feeding trials in the laboratory or small-plot trials in the field.

87. Conversely, it is argued that 'feeding' trials might create such artificial conditions, removing choice of food, etc. that they greatly overestimate effects that would occur in an ecosystem. An example of such a 'dilution' of effect is that of the monarch butterfly in North America, for which damaging effects of Bt maize pollen, found in the laboratory, were subsequently shown unlikely to occur in the ecosystem. There were several reasons for this: much of the insect's food plant occurred outside Bt maize fields, the seasonal timing of the insect and the release of pollen only partly overlapped and the actual exposure of the insect to the toxin was lower in maize fields than in the laboratory. A lay article Butterflies and Bt corn: allowing science to guide decisions, published by several US universities and the USDA provides a summary of the study; scientific papers are assembled in the journal Proc. Natl. Acad. Sci. USA, vol. 98, 2001. The results of many other feeding experiments would similarly be 'diluted' (this reviewer believes) when examined in the context of the ecosystem.

88. The general absence of ecological standards and comparators. As discussed in the introductory notes, and in relation to herbicide-tolerance (Q.6), a context and comparator are vital to any assessment, and as in the case of herbicide-tolerance, they have rarely been taken fully into

account when assessing Bt crops. Assessments purporting to show harm, for instance, should compare the effects of the Bt crop with an existing practice or standard, such as current pesticide management, or options using application of Bt preparations (for which there appears little ecological information). As for assessment of GMHT crops, there is no definition or set of criteria for an acceptable, resilient and sustainable ecosystem. It should be feasible for scientists to define such criteria, which would be generic but with location-specific elements. A recent case study of Bt maize in Kenya shows what can be done quickly by combining global and location-specific knowledge (Hilbeck A & Andow DA, eds. 2004. Environmental risk assessment of genetically modified organisms. Vol 1, A case study of Bt maize in Kenya. CABI Publishing).

89. Mitigation. It much more easy to confine maize within cropped fields than it is oilseed rape or beet, for example, so the ecological risks are mainly to do with the in-field food web and with any wide ranging organisms that visit the field (i.e. there are no risks in much of Europe through ferals and volunteers). To plan a mitigation strategy needs understanding of the existing state of the food web. In the UK Farm Scale Evaluations (see Notes), which used >60 fields of GMHT, not Bt, maize, the maize fields supported a small set of organisms compared to the organisms in oilseed rape and beet. This was so because the persistent herbicides then used on maize severely reduced the arable plants available for the insects to eat. Moreover, only a few species in the above-ground food web were found on the maize itself. If maize is adopted over wide areas of a country where it had not been grown, that itself would affect biodiversity whether or not it was Bt maize. Any additional effect of Bt should be seen in relation to the effect caused by the introduction of maize as a crop species. However, if Bt maize was found to harm the in-field biodiversity, it should be feasible to reverse the effect by growing different crops in a rotation. Perhaps the main uncertainty is the effect of Bt maize on the soil food web. As indicated, the ECOGEN project should inform on this. Effects in the soil would be harder to monitor because it is very difficult to assess soil biodiversity, particularly among bacteria and fungi. Nevertheless, any adverse effects should be mitigatable through a rotation.

90. Q.3 addresses a complex topic. The following is offered in summary.

- The evidence is insufficient to confirm whether widespread cultivation of Bt crops in Europe would affect non-target organisms.
- The weight of evidence from contained experiments and small-plot field experiments generally favours the view that little immediate toxic effect of Bt maize on the food web will occur in the field; but the evidence admits the possibility of chronic effects following long-term exposure to Bt maize.
- The question could only be answered by long-term study of the effects of Bt maize and a comparator on the complex interactions among non-target organisms; it would be difficult to see how that would be done without intensely monitored, field-scale experimental plantings in Europe.
- Criteria for resilient food webs would need to be proposed and agreed as a standard for any new research: without such criteria, progress is impossible.

**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?**

**Dr. Andow**

91. Evidence for resistance. Widespread non-biotech applications of Bt toxins have resulted in the Bt-resistant target pest organisms under field conditions.<sup>25</sup> Probably the best studied example is diamondback moth on cabbage, which has developed field resistance to Bt insecticide applications in several countries, including the US (Hawaii), Japan, Philippines and elsewhere.

92. There is strong evidence that resistance will develop in the field to any insecticide applied uniformly over wide areas for a long enough period of time.<sup>26</sup> This has been a scientific consensus since the 1980s.

93. Bt-resistant target organisms have been found – pink bollworm (*Pectinophora gossypiella*) to Bt cotton in the field,<sup>27</sup> but this has not yet led to field failures.<sup>28</sup>

94. Evolutionary theory predicts that the evolution of resistance will proceed as directional selection, at least up to the point resistance becomes common. This means that resistance in the field cannot be prevented, it can only be delayed.

95. There is a scientific consensus that resistance to Bt maize is inevitable,<sup>29</sup> even though it still has not been detected in the field.<sup>30</sup> This is based on the preceding empirical and theoretical evidence.

96. Generally speaking, of all of the potential environmental risks of transgenic Bt crops, it can be said that resistance in the target pests is a real, tangible risk, while risks associated with gene flow and risks to non-target organisms are mostly only potential risks.

97. There are several risk management options for Bt maize, but they are all concerned with reducing the selective advantage of resistant alleles in populations of the target species.<sup>31</sup>

98. The most widely used resistance management strategy for Bt maize (and all Bt crops) has been the high-dose/ refuge strategy.<sup>32</sup> This has been used by the US Environmental Protection

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<sup>25</sup> Tabashnik, B.E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Ann. Rev. Entomol.*, 39, 47-79.

<sup>26</sup> NRC (National Research Council). (1986). *Pesticide resistance: strategies and tactics for management*. National Academy Press, Washington, DC.

<sup>27</sup> Tabashnik, B.E., Patin, A.L., Dennehy, T.J., Liu, Y.B., Carrière, Y., Sims, M.A. & Antilla, L. (2000). Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. *PNAS*, 97, 12980-12984.

<sup>28</sup> Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. & Zhao J.-Z. (2003). Insect resistance to transgenic Bt crops: Lesson from the laboratory and field. *J. Econ. Entomol.*, 96, 1031-1038.

<sup>29</sup> Tabashnik, B.E. (1994). Evolution of resistance to *Bacillus thuringiensis*. *Ann. Rev. Entomol.*, 39, 47-79. Gould, F. (1998). Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Ann. Rev. Ent.*, 43, 701-726. Andow, D.A. 2001. Resisting resistance to *Bt* corn, pp. 99-124. In D.K. Letourneau and B.E. Burrows (eds.), *Genetically engineered organisms: Assessing environmental and human health effects*. CRC Press, Boca Raton, FL.

<sup>30</sup> Bourguet, D., Chaufaux, J., Séguin M., Buisson, C., Hinton, J.L., Stodola, T.J., Porter, P., Cronholm, G., Buschman, L.L. & Andow, D.A. (2003). Frequency of alleles conferring resistance to *Bt* maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. *Theor. Appl. Genet.*, 106, 1225-1233.

<sup>31</sup> Gould, F. (1998). Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Ann. Rev. Ent.*, 43, 701-726.

<sup>32</sup> A major factor determining resistance management is the "dose" of the Bt crop. Dose is defined as a concentration of Cry toxin in the Bt crop plant in relation to the expression of the resistance phenotypes. For a high dose, the concentration of Cry toxin must be sufficiently high that the plant tissue is toxic enough so that resistance is functionally recessive (Fig. B1, concentration **HD** or higher, Taylor & Georghiou 1979; Tabashnik & Croft 1982). Any other concentration is a low dose. A high dose is desired for resistance management because resistance can be delayed more readily than for a low dose by using the high-dose/ refuge resistance management strategy. A refuge is an area where the pest is not subjected to selection from the Cry toxin and can produce a viable population. The same crop plant without the Bt gene can function as a refuge as long as Bt insecticides are not applied to it. Three conditions are essential to the success of the high-dose/ refuge strategy: resistance is recessive, resistance is rare, and there is sufficient intermating between adults coming from refuges and Bt fields.

The recessivity of resistance is determined by complex interactions (Tabashnik & Croft 1982). If resistance is determined by allelic variation at a single locus, with *R* designating the resistance allele and *S* the susceptible allele, dominance of the *R* allele is related to both the mechanism of resistance in the insect and the toxin concentration in the plant (Fig. 1, appendix to question). For example, if resistance in the insect is related to a change in a receptor in the midgut lining, resistance may be physiologically recessive (Fig. 1a, appendix to question), while in other cases, the dose-mortality curve for *RS* heterozygotes may be physiologically co-dominant (Fig. 1b, appendix to question) or dominant. In both cases, the toxin concentration in the plant will also determine whether the *R* allele functions as a recessive allele (**HD** in Fig 1a and 1b, appendix to question). If the concentration is not high enough relative to the dose-mortality curve for the *RS* heterozygote, the Bt crop would be a low dose crop. Clearly, high dose cannot be determined if resistance has not been observed in the target pest.

Recessive resistance to Bt cotton (Cry1Ac) has been found in cotton budworm (*Heliothis virescens* (F.), Gould *et al.* 1997, Gahan *et al.* 2001), cotton bollworm (*Helicoverpa armigera* (Hübner), Akhurst *et al.* 2003) and pink bollworm (*Pectinophora gossypiella* (Saunders), Tabashnik *et al.* 2000), but no resistance has been found in cotton bollworm (*Helicoverpa zea* (Boddie)). Resistance to Bt maize, which is the most widely grown Bt crop, has not been found in the widely distributed European corn borer (*Ostrinia nubilalis* (Hübner), Bourguet *et al.* 2003) or in southwestern corn borer (*Diatraea grandiosella* (Dyar)). Resistance has been found in the beetle *Chrysomela tremulae* F. to Bt poplar (Genissel *et al.*, 2003), but not in rice stemborer (*Scirpophaga incertulas* (Walker), Bentur *et al.* 2000) to Bt rice. Neither Bt poplar nor Bt rice is planted commercially at this time.

There have been several attempts to designate a high dose without reference to a resistance allele, because the observed resistance alleles may not be the only ones and resistance has not been observed in some species. The EPA-SAP (1998) has suggested that 25 times the the concentration of toxin at which 99% of the individuals in a susceptible population die (LC<sub>99</sub>, lethal concentration 99) may function as a high dose. Caprio *et al.* (2000) argue that 50 times the LC<sub>99</sub> is a better supported by the published data on insecticide resistance. The value of the EPA-SAP (or Caprio *et al.* 2000) standard is that it is readily operationalized for transgenic Bt crops. The fundamental problem with this approach, however, is that it does not anticipate novel resistance mechanisms and may contribute a false sense of security about the validity of the high-dose assumption.

If resistance is recessive, it challenging to measure when rare. As a rule of thumb, for the high-dose/ refuge strategy to be effective, the frequency of resistance should be <0.001 (Roush & Miller 1986). This implies that resistant phenotypes will be extremely rare, <1 x 10<sup>-6</sup>, and that more than 10<sup>6</sup> individuals from natural populations must be screened to estimate such low allele frequencies.

Refuges are areas of habitat within normal dispersal distance of the target species from a Bt habitat where the *R* and *S* alleles have similar fitness. In addition, an effective refuge must be able to support a viable population of *SS* homozygotes in the transgenic landscape. A refuge must be within normal dispersal distances so that there is sufficient mixing and mating between individuals emerging in the refuge and Bt field. A refuge can be any habitat where the target species occurs, including the non-*Bt* crop, other crops, and other non-crop plants, as long as the *R* allele does not have a selective advantage in these fields. If the refuge areas cannot support a viable population of *SS* homozygotes, then the population tends toward extinction, and there are insufficient *SS* individuals available to delay resistance evolution (Ives & Andow 2002).

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Agency (US-EPA). For Bt maize, it is generally accepted that a 20% refuge is needed for the high-dose/ refuge strategy to be effective.<sup>33</sup>

99. Low-dose Bt events will require a greater refuge. There is no scientific consensus as to how big a refuge is needed. One approach, used by the US-EPA is to require a 20% refuge during the initial period of use, when market penetration remains low. This may provide enough time for the scientific evidence to accumulate so that an appropriate refuge size can be determined. A different approach was used by Australia, where a very large refuge was required initially, which was gradually reduced over time to a 70% refuge.

100. Additional resistance management strategies may become possible to develop after resistance in the target pest is identified and characterized.

101. The efficacy of the high-dose/refuge strategy cannot be assessed empirically in the field. Indeed, it would probably be unethical to conduct such a field experiment because it would be necessary to have a positive control treatment where resistance was encouraged. Resistant insects in such a control treatment could escape, leading to field failures that undermine the efficacy of the high-dose/ refuge strategy.

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stem borer, *Scirpophaga incertulas* (Lepidoptera: Pyralidae). J. Econ. Entomol., 93, 1515-1521. Bourguet, D., Chaufaux, J., Séguin, M., Buisson, C., Hinton, J.L., Stodola, T.J., Porter, P., Cronholm, G., Buschman, L.L. & Andow, D.A. (2003). Frequency of alleles conferring resistance to Bt maize in French and US corn belt populations of the European corn borer, *Ostrinia nubilalis*. Theor. Appl. Genet., 106, 1225-1233. Caprio, M.A., Sumerford, D.V. & Sims, S.R. (2000). Evaluating transgenic plants for suitability in pest and resistance management programs. In: *Field manual of techniques in invertebrate pathology* (eds. Lacey, L.A. & Kaya, H.K.). Kluwer Academic Publishers, Amsterdam, pp. 805-828. EPA-SAP (US Environmental Protection Agency – Scientific Advisory Panel). (1998). Transmittal of the final report of the FIFRA Scientific Advisory Panel Subpanel on *Bacillus thuringiensis* (Bt) plant-pesticides and resistance management. Meeting held on February 9-10, 1998. Report dated, April 28, 1998. (Docket Number: OPPTS-00231). Gahan, L. J., Gould, F., & Heckel, D. G. (2001). Identification of a gene associated with Bt resistance in *Heliothis virescens*. Science, 293, 857-860. Génissel, A., Augustin, S., Courtin, C., Pilate, G., Lorme, P. & Bourguet, D. (2003). Initial frequency of alleles conferring resistance to *Bacillus thuringiensis* poplar in a field population of *Chrysomela tremulae*. Proc. R. Soc. Ser. B.-Bio., 270, 791-797. Gould, F., Anderson, A., Jones, A., Sumerford, D., Heckel, D.G., Lopez, J., Micinski, S., Leonard, R. & Laster, M. (1997). Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. PNAS, 94, 3519-3523. Ives, A.R. & Andow, D.A. (2002). Evolution of resistance to Bt crops: Directional selection in structured environments. Ecol. Lett., 5, 792-801. Roush, R.T. & Miller, G.L. (1986). Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol., 79, 293-298. Tabashnik, B.E., Patin, A.L., Dennehy, T.J., Liu, Y.B., Carrière, Y., Sims, M.A. & Antilla, L. (2000). Frequency of resistance to *Bacillus thuringiensis* in field populations of pink bollworm. PNAS, 97, 12980-12984. Tabashnik, B.E. & Croft, B.A. (1982). Managing pesticide resistance in crop-arthropod complexes: Interactions between biological and operational factors. Environ. Entomol., 11, 1137-1144. Taylor, C.E. & Georghiou, G.P. (1979). Suppression of insecticide resistance by alteration of gene dominance and migration. J. Econ. Entomol., 72, 105-109.

<sup>33</sup> EPA /US Environmental Protection Agency). (2001). Biopesticides registration action document – *Bacillus thuringiensis* plant-incorporated protectants. 16 October 2001. [WWW document] URL [http://www.epa.gov/pesticides/biopesticides/pips/bt\\_brad.htm](http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm) (last accessed 23 November 2004).

102. Some greenhouse experiments have confirmed the efficacy of the high-dose/refuge strategy.<sup>34</sup>
103. Theory has predicted that the high-dose/ refuge strategy will be efficacious when resistance allele frequency  $<0.001$ ,<sup>35</sup> resistance is recessive, and there is sufficient adult movement between the refuge and Bt fields to ensure matings between resistant and susceptible phenotypes.
104. These assumptions have been confirmed scientifically for European corn borer (*Ostrinia nubilalis*) in the northern US corn belt. These assumptions are likely for European corn borer in the southern US and in western Europe. They have not been confirmed for southwestern corn borer (*Diatraea grandiosella*) in the southern US, for European corn borer in eastern Europe, or for Mediterranean corn borer (*Sesamia nonagrioides*) in southern Europe. Hence, it can be concluded that in some circumstances it is possible to predict that the high-dose/ refuge strategy should be efficacious. It is possible that the absence of detection of field resistance to Bt maize in the US is partially attributed to the efficacy of the high-dose/ refuge strategy.

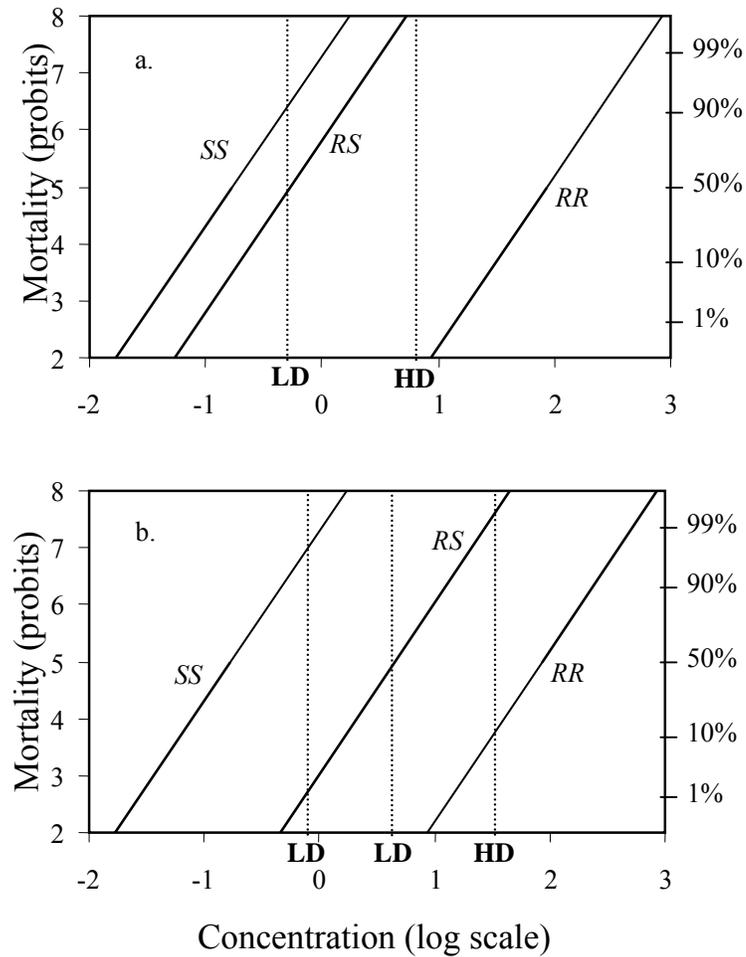
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<sup>34</sup> Shelton, A.M., Tang, J.D., Roush, R.T, Metz, T.D. & Earle, E.D. (2000). Field tests on managing resistance to Bt engineered plants. *Nature Biotechnology*, 18, 339-342.

<sup>35</sup> Roush, R.T. & Miller, G.L. (1986). Considerations for design of insecticide resistance monitoring programs. *J. Econ. Entomol.*, 79, 293-298.

**Appendix**

Figure 1. High dose (HD) and low dose (LD) concentrations (dotted vertical lines) in relation to hypothetical mortality of *SS*, *SR*, and *RR* insect genotypes as a function of *Bt* crystal protein concentration (solid diagonal lines). a. *R* allele is physiologically recessive, so *SS* and *RS* mortality is similar. b. *R* allele confers an intermediate, co-dominant level of physiological resistance, so *RS* mortality is about midway between *SS* and *RR* mortality.



**Dr. Squire**

105. The emergence of resistance by pest insects to pesticides differs widely from context to context depending on factors such as the exposure to and strength of the toxin, the movement of insect populations from areas where the pesticide is not applied, and the genetics and mating system of the insect. Resistance to Bt crops has occurred and is influenced by the 'dose' of toxin delivered to the pest and the genetic nature of the pest, among other factors. Because of long-standing scientific interest in pest resistance generally, knowledge has been imported from that body of work to construct models for the evolution and mitigation of Bt resistance. The processes involved in Bt resistance and its management are generally appreciated by scientists, and mitigation strategies that have a strong scientific basis have been considered. By the late 1990s, mathematical models of the population dynamics of resistant and susceptible biotypes were being used to estimate the number of years for which the GM trait would remain effective in the face of genetic adaptation by the pest. The point is that sound scientific knowledge has been applied to this problem for several years.

106. A strategy for mitigation needs protocols for both pre-emptive management, testing for resistance and managing it if found. The strategies might involve providing refuges in or near the crop where resistant individuals do not have an advantage over resistant ones, periodically controlling the pest by other means and growing different 'types' of Bt maize, or Bt and non-Bt maize, either together or in sequence. Mitigation will be much easier to implement where the existing ethos is sympathetic to integrated pest management based on genetic and ecological principles. A useful, recent summary of the topic, much of it in layman's terms, directed at Bt maize in Kenya but based on generic arguments, is given by: Fitt GP et al., (2004). Resistance risks and management associated with Bt maize in Kenya. In Hilbeck A & Andow DA, eds. 2004. Environmental risk assessment of genetically modified organisms. Vol 1, A case study of Bt maize in Kenya. CABI Publishing.

**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 efficacy?**

**Dr. Nutti**

107. 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.

**Dr. Squire**

108. (This response does not address the part of this question relating to humans. Toxicity was considered as part of the Response to Q.3 and some of the argument here is repeated.) Assessing a product's toxicity to ecological processes is usually more complicated than assessing the toxicity of, for example, a feedstuff for a domestic or farm animal. The domestic or farm animal may have the food under test as its sole or main diet in reality, whereas non-target organisms generally have a wider choice of food throughout the habitat. Also, the expression of a toxin in a plant and its effect on animals eating the plant are influenced by local growing conditions, the weather, the behaviour or the local animals, etc., all of which differ between sites. Therefore making inferences about toxicity to animals in one part of the world (e.g. a maize field in a European country) from information in a constrained experiment, or field experience in another part of the world, should be done with great caution. The contention that nothing adverse has occurred when growing Bt maize in, say, North

America, should not be taken as definitive evidence by itself that nothing will occur in Europe, where the organisms and their interactions are different.

109. Feeding experiments in which organisms are exposed to live or dead Bt plant matter, and have no other choice of food, should be viewed as providing information only on the possibility that the Bt plant might be toxic to the animals in the field. Many of the studies in contained experimental systems are of this type. Some do and others do not indicate harm occurred, but none should by themselves be taken to indicate that harm will necessarily occur in the field. (Several major crop species would fail toxicity tests of this type.) Many of the plot-scale experiments examined in EC-149 were criticised by the authors of that review for a range of reasons, but taken as a whole, experiments at the scale of small field plots and one season's exposure indicate no acute toxicity to animals other than the pest.

110. Evidence, either way, of harm to animals of growing Bt crops in the field in Europe is therefore inconclusive at present. The question could be approached by a range of experimental studies over longer periods, but the results would need to be compared against the effects of other crop species on the animals, and against independent criteria defining a normal or healthy state for the animals in question (as for Q.3).

**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 the International Plant Convention's (IPPC) International Standard for Phytosanitary Measures (ISPM) 11 (EC-130)?**

- (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?**
- (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 species; infertile wild relatives; seed survival in relevant pedoclimatic conditions; the reproduction biology of the species; or other factors?**
- (c) **Is this potential different for biotech crops tolerant to two wide-spectrum herbicides? Please explain.**
- (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?**
- (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?**

**Dr. Nutti**

111. According to the International Plant Convention's (IPPC) International Standard for Phytosanitary Measures (ISPM) 11 (EC-130), the definition of pest is "Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products". In my opinion, herbicide tolerant crops can not be qualified as "pest", according to this definition.

**Dr. Andow**

112. I will provide a theoretical response to the main question and address only part (e) of the subparts of this question. I will use the seven kingdom taxonomy here and throughout in discussing organisms.

113. Persistence of herbicide tolerant crops (whether biotech or developed through mutagenesis) will be determined by the relative fitness of the crop with the trait and the degree of release of the trait. The only way that a GMHT would be more persistent than a non-GMHT crop would be if it had a higher relative fitness and/or it was released at a higher rate. Both factors would have to be assessed on a case-specific basis. For GMHT maize, cotton, soybean and beet, the question is moot because there are no comparably good non-GMHT varieties. For GMHT oilseed rape, evidence from Canada suggests that the GMHT varieties are used more commonly than the non-GMHT varieties, thus there is some scientific evidence to suggest that GMHT oilseed rape may be more persistent in the agricultural or non-agricultural environment than their conventional HT oilseed rape.

114. A similar argument holds for persistence of herbicide tolerant crops (whether biotech or developed through mutagenesis) compared to their conventional counterparts. When examining release rate, however, the relevant release rate for the conventional counterpart is that of a single variety.

115. Annex 2 of ISPM 11 specifies "Phytosanitary risks from LMOs may result from certain traits introduced into the organism, such as those that increase the potential for establishment and spread, or from inserted gene sequences that do not alter the pest characteristics of the organism by that might act independently of the organism or have unintended consequences." Thus phytosanitary effects of the transgene outside of the original organism of introduction are covered by ISPM 11.

116. Annex 2 elaborates on this further in the next clause, which states "In cases of phytosanitary risks related to gene flow, the LMO is acting more as a potential vector or pathway for introduction of a genetic construct of phytosanitary concern rather than as a pest in and of itself. Therefore the term "pest" should be understood to include the potential of an LMO to act as a vector or pathway for introduction of a gene presenting a potential phytosanitary risk." Phytosanitary risks associated with gene flow and persistence are covered by ISPM 11.

117. Moreover, Annex 3 "Determining the potential for a living modified organism to be a pest", specifies that "potential phytosanitary risks for LMOs may include: a. Changes in adaptive characteristics which may increase the potential for introduction or spread, ... b. Adverse effects of gene flow or gene transfer. This indicates that the potential for introduction and spread are a part of phytosanitary risk, as well as adverse effects of gene flow.

118. From the perspective of gene flow and persistence, GMHT crops may qualify as a potential pest according to ISPM 11, as long as they can be shown to be released at a higher rate and/or have a selective advantage compared to a conventional counterpart.

119. Of course it is possible for a GMHT crop to qualify as a potential pest according to ISPM 11 if persistence is expected to be the same as a conventional counterpart. This would occur if it potentially resulted directly or indirectly in some adverse effect that was different from the conventional counterpart. For example, if contamination of conventional crop germplasm (related to the "coexistence" issue) were considered an injury to the conventional plant, then there would be a reason to believe that all GMHT crops can be considered potential pests under ISPM 11. All other potential pest risks would probably have to be considered on a case by case basis.

120. ISPM 11 is ambiguous whether all possible effects of GMHT crops can be considered phytosanitary risks. The definition of "pest" is given on page 6 of ISPM 11 as "Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products [FAO, 1990; revised FAO, 1995; IPPC, 1997]" The concept of "injurious to plants includes environmental risks, as indicated in supplementary text S1.

121. Annex 2, which covers the scope of IPPC for LMOs states "PRA may constitute only a portion of the overall risk analysis for import and release of a LMO. For example, countries may require the assessment of risks to human or animal health, or to the environment, beyond that covered by the IPPC." This is a clear acknowledgement that there are some environmental risks that are not covered by the IPPC.

122. Annex 1 addresses the scope of the IPPC in regard to environmental risk. This text is reproduced here:

123. "The full range of pests covered by the IPPC extends beyond pests directly affecting cultivated plants. The coverage of the IPPC definition of plant pests includes weeds and other species that have indirect effects on plants, and the Convention applies to the protection of wild flora. The scope of the IPPC also extends to organisms which are pests because they:

– *directly affect uncultivated/unmanaged plants*

124. Introduction of these pests may have few commercial consequences, and therefore they have been less likely to be evaluated, regulated and/or placed under official control. An example of this type of pest is Dutch elm disease (*Ophiostoma novo-ulmi*).

– *indirectly affect plants*

125. In addition to pests that directly affect host plants, there are those, like most weeds/invasive plants, which affect plants primarily by other processes such as competition (e.g. for cultivated plants: Canada thistle (*Cirsium arvense*) [weed of agricultural crops], or for uncultivated/unmanaged plants: Purple loosestrife (*Lythrum salicaria*) [competitor in natural and semi-natural habitats]).

– *indirectly affect plants through effects on other organisms*

126. Some pests may primarily affect other organisms, but thereby cause deleterious effects on plant species, or plant health in habitats or ecosystems. Examples include parasites of beneficial organisms, such as biological control agents.

127. To protect the environment and biological diversity without creating disguised barriers to trade, environmental risks and risks to biological diversity should be analyzed in a PRA."

128. In considering indirect effects, Annex 1 addresses "indirect effects on plants" and "indirect effects on plant through effects on other organisms." Indirect effects are not defined, but the two examples provided are two of the five fundamental two-species interactions in ecological science, consumption (parasite-host or predator-prey or herbivore-plant), competition, mutualism, amensalism, and commensalism. Thus, indirect effects occur via any combination or pathway of these to-species interactions. This is the standard interpretation of the concept of indirect interactions which give rise to indirect effects in the field of ecology. As the IPPC is science-based, I assume that standard scientific usage is appropriate for interpreting key terms taken from science.

129. Under this interpretation (and other interpretations as well), the effects of the herbicides applied to the GMHT crops is not covered under Annex 1 and can not be considered a phytosanitary risk. Herbicide use cannot be considered a direct or indirect effect of the GMHT crop, because humans apply the herbicides. It is inaccurate to say that this is an indirect effect of the GMHT crop through effects on other organisms. The GMHT crop does not affect humans to apply herbicides. Causality works the other way. Humans affect the distribution of the GMHT crops and at the same time affect herbicide use. Thus, the effects of herbicide use on GMHT crops is not covered under Annex 1.

130. Section 2.3 of ISPM 11 covers "Assessment of potential economic consequences" and also covers environmental risk. Under 2.3.1.2, indirect pest effects are considered. This is a list of potential endpoints to consider given that it is an indirect effect. Thus, section 2.3 does not provide an interpretation to cover the effects of herbicides applied to GMHT crops as a phytosanitary risk.

131. It would be disingenuous to suggest that herbicide effects should be considered as if they were independent of the use of a GMHT crop. While it may be true that the environmental effects of herbicides are regulated under independent regulatory structures, this does not change the fact that they are used part and parcel with GMHT crops because that is their intended use. Thus GMHT crops almost always will be accompanied by the use of the specific herbicide.

132. The only ecologically consistent position would be to identify humans as the potential pest. This, however, is absurd under ISPM 11.

133. Thus I conclude that GMHT 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 GMHT crops can be considered phytosanitary risks.

(e) Post-market monitoring and data collection:

134. Section 3.6.1 of ISPM 11 describes the monitoring and review of phytosanitary measures. This concentrates on the principle of "modification", and is repeated here.

**3.6.1 Monitoring and review of phytosanitary measures**

The principle of "modification" states: "As conditions change, and as new facts become available, phytosanitary measures shall be modified promptly, either by inclusion of prohibitions, restrictions or requirements necessary for their success, or by removal of those found to be unnecessary" (ISPM N° 1: *Principles of plant quarantine as related to international trade*).

Thus, the implementation of particular phytosanitary measures should not be considered to be permanent. After application, the success of the measures in achieving their aim should be determined by monitoring during use. This is often achieved by inspection of the commodity on arrival, noting any interceptions or any entries of the pest to the PRA area. The information supporting the pest risk analysis should be periodically reviewed to ensure that any new information that becomes available does not invalidate the decision taken.

135. This suggests that an important part of the risk management measures applied to GMHT crops should include a process to review the necessity of the management measures. Given that many ecological effects are scale-dependent, manifesting more readily at larger spatial and temporal scales, some management measures could be linked to the spatial extent of use of the GMHT crop. If the spatial extent of use is not great enough, then certain management measures could be considered unnecessary. Alternatively, if the GMHT trait persists in a given locality for long enough, additional management measures could become necessary.

136. In a similar fashion, after certain time and area thresholds have been exceeded, it would be reasonable to review management measures to determine if the measures need to be dropped, retained or strengthened. Such thresholds should be agreed upon in advance.

137. Finally, for precautionary management measures, there should be certain time and area thresholds specified which would trigger a review to determine if sufficient information had accumulated to merit a reduction in the level of precaution. Presumably the precautionary management measures would provide data from which it would be possible to assess their necessity.

#### **Dr. Snow**

138. Note: I use the terms herbicide resistance and herbicide tolerance interchangeably. Weed scientists generally use the term resistance in this context to distinguish between a newly evolved condition and an existing level of tolerance that has always been present. Biotech companies prefer to use the term tolerant for transgenic crops that have new genes for protection from herbicides.

139. ISPM 11 definition of a pest (explained further in Annexes 1-3 of ISPM No. 11):

- "Any species, strain, or biotype of plant. ... injurious to plants or plant products,"
- "... many organisms indirectly affecting plants also satisfy this definition (such as weeds/invasive plants)"
- Annex 3 mentions LMOs with increased potential for spread due to ... "pesticide (including herbicide) resistance or tolerance"

140. In summary, 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. These plants could become more abundant if they gain one or more types of herbicide resistance from the crop. This can occur when the herbicide in question is used extensively (e.g., glyphosate) and herbicide-resistant volunteers become much more difficult to control.

141. The magnitude of the problem, if and when there is a problem, depends on whether resistant volunteers and related weeds can be killed using other herbicides, crop rotation, and/or tilling, and on what amount of effort is needed to keep these plants from harming crop yields (e.g., Warwick et al. 1999, Friesen et al. 2003). This problem varies among species of crops and weeds that occur in different countries. Brassica rapa is known to hybridize with oilseed rape in Europe, but its abundance is usually kept under control by tilling and herbicide applications.

142. I conclude that there is no evidence to suggest that herbicide-resistant plants are more persistent in non-agricultural areas where herbicides are not used (e.g., Crawley et al. 2001), and there is no scientific reason to expect that this could happen. Also, each type of herbicide-tolerant crop should be considered individually – it is not possible to generalize across all herbicide-tolerant crops.

143. ANSWERS 6a. and 6b: Note: I assume that this question refers to plants that are cultivated by farmers, rather than seeds that are imported exclusively for processing.

#### Routes of dispersal

144. Seeds – Genes for herbicide resistance, whether transgenes or not, can spread by means of seed movement. Herbicide-tolerant crop plants can easily spread by seed dispersal that occurs during handling, transport, spillage. Depending on the crop, seeds from volunteers, feral populations, and weedy relatives of the crop also disperse widely by wind, water, farm vehicles, and the transport of seeds, forage, and manure.

145. Pollen – Like other crop genes, transgenes for herbicide tolerance can spread to sexually-compatible crops and wild or weedy relatives that occur nearby by cross-pollination. Therefore, the potential for spread and establishment of transgenes is greater when similar crops or wild/weedy relatives occur nearby.

146. Pollen-mediated transfer of crop genes to other fields and to sexually-compatible wild relatives occurs more frequently in crops that typically cross-pollinate, such as maize and to a lesser extent oilseed rape, than in soybean, which is mostly self-pollinated. With respect to oilseed rape, the weed Brassica rapa is an obligate outcrosser that is known to hybridize with the crop (e.g., Jeorgensen and Andersen 1994).

147. Transgenic tolerances to both glyphosate (Round-Up) and glufosinate (Liberty, Basta) are inherited as dominant, Mendelian traits. Some transgenic varieties may have more than one insertions of a gene for herbicide tolerance, while others have just one. A plant needs just one copy of the gene at one insertion site to be tolerant of the herbicide in question. This means that pollen from the crop can transfer the herbicide tolerance trait to seeds in other farmers' fields and to seeds of volunteer crop plants, related weeds, or wild relatives. When different crop varieties have different genes for herbicide tolerance, weed populations can acquire several types of herbicide resistance genes, as shown by Hall et al. (2001) for weedy, volunteer oilseed rape in Canada.

#### Potential for establishment

148. Some crops establish volunteer and feral populations more easily than others, depending largely on how thoroughly domesticated the crop is. Oilseed rape is not highly domesticated in the sense that it can survive as free-living, volunteer populations that can re-seed from year to year (e.g., Pessel et al. 2001). Also, viable seeds of oilseed rape can persist in the soil for several years, and then germinate and reproduce (Simard et al. 2002). In contrast, maize does not establish feral populations

in Europe. At most, maize plants can be present as volunteers in the year following the crop, but they do not establish problematic, long-term populations.

149. Hybridization with sexually-compatible weedy relatives can further allow transgenes to spread and persist in and around agricultural fields. Even when first-generation offspring between a crop and a weed are inferior to normal weeds, crop genes can often become incorporated into the local gene pool of the weed following further cross-pollination. If progeny from these crosses are completely infertile, they will not produce seeds and they will not be a problem unless they establish clonal, vegetatively propagated populations, as in perennial grasses.

#### Further spread after establishment

150. Frequencies of specific crop genes in free-living plant populations depend on their rates of introduction and also their effects on plant fitness (i.e, relative survival and reproduction). Unlike some types of nontransgenic herbicide tolerance, the transgenes that confer tolerance to glyphosate and glufosinate are not expected to have any negative effects on crop yields or the fitness of crop relatives (e.g., Snow et al. 1998).

151. 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 (e.g., Warwick et al. 1999, WSSA 2005).

152. ANSWER 6c: In principal, the potential for the establishment and spread of herbicide-tolerant plants is similar for nontransgenic vs. transgenic crops that have genes for these traits. Rates of establishment and spread of herbicide-tolerant crops are affected by whether the genes for herbicide resistance show dominant or recessive inheritance, whether the resistance genes are associated with fitness costs (i.e., reduced survival or reproduction), and the extent to which the plants are exposed to the herbicide(s) in question. Transgenes that confer resistance to glyphosate or glufosinate show dominant inheritance and they are not likely to be associated with fitness costs (e.g., Snow et al. 1998).

153. In answers 6a and 6b, I discuss how biotech crops with resistance to either of these two wide-spectrum herbicides might contribute to the spread and establishment of herbicide-resistant weeds. This can happen by gene flow to related weeds, and also by another mechanism – spontaneous selection for weeds that are naturally resistant to the herbicide. The second mechanism has occurred in only a few species for the two herbicides in question. Once a gene for glyphosate or glufosinate resistance is present, is likely to increase in frequency in compatible weeds and volunteer populations that are exposed to these herbicides. The more the herbicide is used, the stronger the selection pressure favoring herbicide-resistant weeds. Glyphosate use is already common and is expected to increase further when crops are bred to have transgenic resistance to this herbicide. Glyphosate is very useful to farmers because it is wide-spectrum, killing most weed species very efficiently, and it is considered to be "environmentally friendly", as compared to other herbicides. Glufosinate is used less widely, but its use also is expected to increase after biotech crops with glufosinate tolerance have been commercialized.

154. ANSWER 6d: Several risk management options are available to mitigate problems that result from plant pest populations that are tolerant of one or more herbicides, but they may or may not be very practical (e.g., Beckie et al. 2004, Devos et al. 2005). One way to prevent herbicide-tolerant weeds from becoming more abundant is to rotate among herbicides with different modes of action

from one year to the next, in a cycle that accounts for the persistence of viable seeds of herbicide-resistant weeds in the soil.

155. Another method for suppressing populations of herbicide-resistant weeds is to use several types of herbicides in tank-mixes each year, before or after a crop is grown, to kill off resistant plants. 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).

156. The efficacy of different management options will vary depending on local conditions and the types of crops and rotation systems that are used. For oilseed rape, which has a long-lived seed bank, seeds of weedy volunteer plants may emerge over a period of at least 4-5 years (Simard et al. 2002).

157. As mentioned above, 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.

158. ANSWER 6e: 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 (e.g., WSSA 2005). 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.

159. 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.

#### **Dr. Squire**

160. The background to this question is that several of the crops have become widespread feral plants or weeds (see Notes, Annex). Oilseed rape has been grown in Europe for centuries, but has become a weed in many parts of Europe following a great increase in its cropped area since the 1970s. It is now a regular and persistent member of the arable and wayside seedbank (buried viable seeds). Oilseed rape is not considered a problem on waysides and is ousted by perennial vegetation such as grass. Within fields, it is treated as part of the broadleaf weed flora. It also contributes to yield of the next oilseed rape crop, but has generally not been considered a problem in this respect, since the quality of oil has generally been similar in the volunteers and crop plants. Similarly weed beet, arising from seeding crop beet, is common in beet-growing areas and brings similar but lesser problems.

161. Are HT plants a potential pest? Oilseed rape and beet are both crops and pests, as are many other crop plants (pests as defined in ISPM-11, page 6). The volunteers or ferals arising from HT crops would be no greater pests than those arising from non-HT crops, unless the specific herbicide was used to favour them as pests, or their presence in the field or in the yield itself had greater significance than the presence of non-HT. Since agriculture began, crops and weeds of the same species have existed side by side and exchanges genes – but this does not mean that volunteer/feral weeds are not pests.

162. More serious problems to do with their persistence as pests will arise if labelling or marketing 'rules' specified that a non-biotech crop had to be of a high degree of purity, i.e. to contain less than, say, 0.9% of GM in the yield.

163. 6a. In the absence of use of the specific herbicide, HT plants would have the same potential as non-HT, as indicated in 6 above. Species would differ as in Table 1. Establishment and spread depends on local conditions in ways that are not clear. For example, feral wayside populations of oilseed rape differ greatly in number in areas studied in France and the UK. Also, wild, weedy relatives differ in their presence and number in different parts of Europe, as do hybridisation rates between crops and between crops and wild relatives. But the essential point is that – in the absence of the specific herbicide to which the plants are tolerant – plants having HT and non-HT traits should act similarly.

164. 6b. Suppose that HT plants were growing in mixed vegetation with non-HT plants of the same and other species, then applying the herbicide would favour the HT plants, lead to greater seed set on HT plants and their increase in the seedbank. This would happen wherever the plants were growing and whether the HT trait was in volunteer/feral plants or had been transferred to wild relatives. Whether such differences occurred in practice would then depend on the how widely the specific herbicides were used (which itself would vary between countries and crops).

165. 6c. In the absence of the specific herbicide, the potential for establishment and spread should be similar for biotech plants tolerant to the wide-spectrum herbicides glufosinate ammonium and glyphosate. The genes conferring tolerance to either should not enable the plants to be better at, say, persisting in the soil. Relative persistence between the two GMHT types would differ if one or both of the herbicides was used. As indicated, this will be highly specific to the agronomy of a farm or region. For instance, in the UK, glyphosate has risen in the last few years to be the second (or maybe first) most widely applied herbicide in arable fields, while glufosinate ammonium is used little in fields or waysides. If GMHT glyphosate tolerant plants were present in fields, then they would be advantaged at this specific time and area, where glufosinate ammonium tolerant plants would not.

166. 6d. Risk management options should be similar to those recommended for non-biotech varieties (oilseed rape and beet), but would need to be more stringently applied if cropping was to ensure the proportion of GM in non-GM remained below a threshold. Establishment can be reduced by –

- Reducing or preventing seed loss to the soil, e.g. by harvesting oilseed rape before too many pods split (not always possible), uprooting flowering beet plants.
- Not cultivating soil after harvest, leaving seeds to germinate on soil and then killing them by cultivation or herbicide spray.
- As part of normal broadleaf weed control, ensure they do not flower and seed in later crops of other species.
- Spread can be reduced by –
- Clearing seed from combine harvesters and other machinery between crops.
- Transporting seed from field to market in sealed containers or vehicles.
- Cutting or spraying roadsides that support large populations of feral oilseed rape, but these practices will impinge on the semi-natural flora.
- Using a pollen barrier to reduce field-to-field gene flow (oilseed rape and maize), such as a strip of flowering crop 50 to 100 m wide between the donor and recipient crops and which is not harvested as part of the recipient crop.

167. The main difficulty in preventing establishment and spread is with oilseed rape, specifically in that it will enter the soil and become dormant even if soil is not cultivated after harvest, it will flower and seed, usually unseen, in a later crop of oilseed rape and it is impractical to remove all seed from farm machinery. Lesser problems occur with weed beet.

168. 6e. Post-market monitoring: 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 (see Notes, paragraph 14), 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.

**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)?**

**Dr. Andow**

169. I will restrict this answer to the following genetically modified herbicide tolerant (GMHT) crops: oilseed rape, fodder beet, cotton, maize, soybean, and sugar beet. I will use the seven kingdom taxonomy in discussing organisms.

Adverse Effects of GMHT Crops:

170. There is abundant evidence that repeated use of a given biotech herbicide tolerant crop would likely result in the evolution of resistance in weeds to the herbicide. While it can be debated if this adverse effect results from repeated use of the GMHT crop or from the repeated use of the herbicide of which the GMHT crop is tolerant, in practice the two are so tightly correlated, and the GMHT crop is expected to have the herbicide applied to it. Hence a risk assessment should consider both as correlated causal factors. In the language before the panel, weed resistance is a risk resulting from the associated changes in agricultural practices.

171. Adverse effects on non-target flora and fauna could arise directly from transgene products, directly from the herbicide compounds, or indirectly through the effects of the transgenic crop or the herbicide on the environment. Although the authors of the Farm-Scale Evaluation (FSE) Trials of GMHT crops in the UK<sup>36</sup> concluded that all adverse non-target effects arise indirectly from the effects

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<sup>36</sup> Brooks et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1847-1862. Champion et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1801-1818. Firbank 2003, Phil. Trans. R. Soc. Lond. B, 358, 1777-1778. Houghton et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1863-1877. Hawes et al. 2003, Phil. Trans. R. Soc. Lond. B, 1899-1913. Heard et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1819-1832. Heard et al. 2003, Trans. R. Soc. Lond. B, 358, 1833-1846. Roy et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1879-1898. Squire et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1779-1799.

of the herbicide on the environment,<sup>37</sup> this highly credible argument is not proved conclusively.<sup>38</sup> In any event, the FSE Trials demonstrate that some, but not all, GMHT crops can have adverse effects on non-target organisms. I believe that it is likely that additional adverse effects on non-target organisms may be reported in the future.

172. Gene flow from a GMHT crop to a weedy relative can create weeds that are more difficult to control with herbicides. This occurred for GMHT oilseed rape in Canada. GMHT maize, cotton and soybean have not yet been grown legally in locations where there are weedy relatives and none occur in Europe, the US, Canada or Argentina, so this risk is not possible in these countries. Beets have wild relatives in Europe, and weedy beets have developed as a consequence of gene flow in the past.

173. Gene flow from a GMHT crop to another crop cultivar can contaminate seed supplies (part of the "coexistence" issue) or reduce genetic diversity in the crop. Contamination is a serious concern, although it can be debated if this adverse effect is on the flora (the crop) or the growers of the crop. It is clear that the harm is to the growers of the crop. Genetic diversity of cotton, maize and soybean is not known to be great in Europe, so this is unlikely to be a concern. It may be possible that there are significant sources of genetic diversity in oilseed rape and beets in Europe.

174. To my knowledge there are no reports of adverse effects on soil micro- or macro-flora or fauna separate from those in the UK-FSE trials. Nor are there any reports of adverse effects on soil dwelling bacteria, algae, or protozoa. However, to my knowledge there have not been any studies of any of these possible effects. The Panel should not infer that the absence of information implies an absence of effect.

175. To my knowledge, all reports have not found adverse effects on flora or fauna from antibiotic resistance genes or gene products. Extensive studies on nptII did not find any adverse effects, and found that any undetected adverse effects would likely be several orders of magnitude smaller than naturally occurring phenomena.

#### Comparison with non-GM HT Crops:

176. There are several ways in which GMHT and non-GMHT crops may differ that are significant to risk to flora and fauna.

177. GMHT traits are typically hemizygous, which means that there is only one copy of the locus in the plant. Normal plant genes occur in two copies, that are either different (heterozygous) or the same (homozygous). Gene flow to wild relatives or other crop varieties will result in hemizygous offspring for GMHT traits, which are likely to exhibit dominant expression. For non-GMHT traits, hybrid offspring are most likely to be heterozygous and may exhibit varying degrees of dominance. This means that whatever the selective advantage of the trait in the original HT plant, it is likely to be similar for GMHT hybrids, but possibly less for non-GMHT hybrids. Because selective advantage is one of key determinants of spread of genes, this would suggest that GMHT traits could be more likely to spread than equivalent non-GMHT traits.<sup>39</sup>

178. The promoters on most GMHT transgenes keep the transgene turned on and expressing at all times. The non-GMHT transgenes are regulated by plant promoters, which may or may not keep the

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<sup>37</sup> Champion et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1801-1818.

<sup>38</sup> Andow 2003, Nature Biotechnology, 21, 1453-1454.

<sup>39</sup> It should be recognized that while the bits of information that comprise this argument can be found in the information before the Panel, the argument itself cannot be found in the information before the Panel.

gene turned on all the time. This may enable the herbicide to be used for a longer period of time on the GMHT crop than the non-GMHT crop. If so, greater degrees of weed reduction may be possible in GMHT than non-GMHT crops, which would result in greater non-target effects and higher selection for weed resistance in more species of weeds.<sup>40</sup>

179. Most of the GMHT crops tolerate glufosinate and glyphosate, while most of the non-GMHT 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.

180. Scale of use has a significant effect on risk.<sup>41</sup> Some GMHT crops have gained significant spatial scales of use that dwarf those of non-GMHT crops. For example, RoundUp Ready<sup>®</sup> 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 FSE trials.

181. If contamination is an adverse effect on flora, then GMHT crops have this risk while non-GMHT crops do not. This is based on the definition of harm, which is premised on distinguishing between GM and non-GM traits.

182. If all of the factors mentioned in paragraphs 177 through 181 are equivalent between the GMHT and non-GMHT crop, then there will be no predicted difference in the risks of adverse effects from the repeated use of a GMHT or non-GMHT crop.

### **Dr. Squire**

183. The difficulties of argument in this topic revolve around what is classed as an adverse effect (Notes, paragraphs 32-35). There are no objective criteria for what is an ideal state and what therefore might be judged an adverse effect against that state. At best, HT cropping has been compared against an existing cropping system. Certainly in Europe, the important and relevant flora and fauna should include those within the managed areas of fields.

184. The Farm Scale Evaluations (FSE) in the UK (Notes, paragraph 14) detected small, but important shifts caused by using GMHT rather than the conventional management, and illustrate issues of context. The difference in weed management rather than the GM or conventional crop plants per se was the effective agent. 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 21<sup>st</sup> century. The flora and fauna of arable fields were important in the national biodiversity as perceived by many people; these flora and fauna had already been severely depleted by intense agriculture, so further depletion was unnecessary and unacceptable.

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<sup>40</sup> It should be recognized that while the bits of information that comprise this argument can be found in the information before the Panel, the argument itself cannot be found in the information before the Panel.

<sup>41</sup> NRC (National Research Council). (1987). *Introduction of recombinant DNA-engineered organisms into the environment: key issues*. National Academy Press, Washington, D.C.

185. In principle, there should be no difference between the effects of GMHT cropping and non-biotech HT cropping, though this has not been compared on a suitable scale. The BRIGHT project (Notes, paragraph 14) made some comparisons of GMHT and non-GM HT crops.

186. A general knowledge of the macro- and micro-fauna in the soil suggests they will be much less sensitive to GMHT cropping than will the above-ground flora and fauna. The FSE and some other work included macrofauna caught on the soil surface, but no large-scale studies of herbicide tolerance have included soil micro-and macro-fauna. (ECOGEN will provide information on soil communities at three field sites for Bt maize.) All evidence from small-scale work in field plots and containers points to the herbicides glyphosate or glufosinate ammonium being much less directly toxic to soil fauna than other agrochemicals and the GMHT plants themselves having no effect on soil organisms different from that of non-GM plants.

**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-1990'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.**
- (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?**

**Dr Nutti**

187. Answer 8a: Beginning in the 1990's, commercial protein-based and DNA-based detection methods have become available. Both methods can be used in a qualitative and a quantitative way. Early DNA-based methods have been mainly designed as qualitative methods only. More recently, there has been a shift towards quantitative PCR methods. Today, most published methods are based on real-time PCR technology that can be operated both in a qualitative and a quantitative way. Some DNA-based methods were designed for the identification of a specific event while others were designed to identify only a certain vector construct or a common genetic element (e.g., 35S promoter). Today, the development of these three different types PCR methods continues. The selection of method depends on the intended purpose. The experience for protein-based methods is similar to the experience with DNA-based methods – there are many commercially available protein-based methods also. These methods can confirm the presence or absence of specific proteins expressed by the different biotech events. These protein-based methods can be applied in both a quantitative and qualitative fashion. The protein based methods, while not event-specific, can identify uniquely most commercial events.

188. Answer 8b: I would like to inform that I am not an expert in this area, and I will list below the information that I could find in order to help the panel, but is important to point out that I will list the references and information founded and not express my personal opinion.

Interlaboratory studies:

189. The International Union of Pure and Applied Chemistry (IUPAC) classify interlaboratory studies into the following three categories:

1. Method performance: Determines the bias and precision of an analytical method.
2. Material performance: Assigns a value and an uncertainty (or reliability) to a characteristic (usually concentration) of a material.
3. Laboratory performance: Permits the evaluation of each participant against preset criteria or criteria estimated from the study itself.

190. Although the procedures regarding statistical data evaluation from these three types of interlaboratory studies may be identical, the use and interpretation of the resulting statistical estimates will be determined by the primary purpose of the study.

Validation of methods:

191. The validation of methods consists of two phases. The first is an in-house validation of all of the parameters except reproducibility. The second is a collaborative trial, the main outcome of which is a measure of the repeatability and reproducibility in order to estimate the transferability of methods between laboratories. Usually a small-scale collaborative trial should be performed to test the ruggedness of a particular method before the expense of organizing a large-scale trial is incurred. In case any improvement of the method or the method description is needed, only limited costs are incurred through the pre-trial, while a failure of a full interlaboratory method validation due to an ambiguous method description is a very costly failure. Implementation of an already validated method in a laboratory needs to include the confirmation that the implemented method performs as well under local conditions as it did in the interlaboratory method validation. A method must be validated using the protocols and reaction conditions under which it will be performed.

192. The Procedural Manual of the Codex Alimentarius Commission, 12th edition, (2001), list the main definitions of the parameters for method validation, although the concepts of a Limit of Detection (LOD), Limit of Quantification (LOQ), and Range of Quantification (ROQ) are not yet explicitly defined by Codex Alimentarius, they are listed below in order to answer this question:

193. Parameters for method validation: Accuracy – The closeness of agreement between the reported result and the accepted reference value. Accuracy describes how close the measured value is to the actual value of a known reference sample.

194. Precision – The closeness of agreement between independent test results obtained under stipulated conditions. Precision describes how well the results agree between repeated analyses of the same material. Less precision is reflected by a larger standard deviation of the combined results than the individual results.

195. Sensitivity – Change in the response divided by the corresponding change in the concentration of a standard (calibration) curve, i.e., the slope of the analytical calibration curve.

196. Specificity – The ability of a method to respond exclusively to the characteristic or analyte. Specificity describes how often the analyte is not detected if the analyte is not....

197. Ruggedness (Robustness) – The ability of a method to resist changes in results when subjected to minor changes in environmental and procedural variables, laboratories, personnel, etc.

198. Applicability – The analytes, matrices, and concentrations for which a method of analysis is validated that may be used satisfactorily.

199. Repeatability – Precision under repeatability conditions. These are conditions in which independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

200. Reproducibility – Precision under reproducibility conditions. These are conditions in which test results are obtained with the same method on identical test items in different laboratories with different equipment and operators.

201. Limit of Detection (LOD) – The lowest amount of analyte in a sample that can be detected with suitable confidence but not necessarily quantified as an exact value as determined by method validation.

202. Limit of Quantification (LOQ) – The lowest amount of analyte in a sample that can be quantified with suitable accuracy and precision as determined by method validation.

203. Range of Quantification (ROQ) – The range within which the amount of analyte in a sample can be quantified with suitable accuracy as determined by method validation. I will assume a PCR detection method for outline the necessary steps. According to ILSI 2001, there are two primary ways in which PCR is used in the detection of GM DNA in plants. These are called quantitative PCR, which yields an estimate of the amount of the specific analyte present, and qualitative PCR, which yields a yes/no answer as to the presence of GM material.

204. The Sample Preparation is considered the first step and it is necessary to take into account the:

- Sampling the consignment of seed or grain to obtain the bulk sample.
- Sampling of the bulk sample to obtain the laboratory sample.
- Subsampling the laboratory sample to obtain the test sample.
- Sampling the meal that results from grinding the test sample to obtain the analytical sample.
- Sampling the DNA solution that results from extraction of the meal sample to obtain the test portion.

205. The DNA extraction is the second step and DNA quantification is the third. I could not find more details for this topic. I also did not find references for identify the differences in validation steps for qualitative and quantitative detection methods but I found that the parameters common to qualitative and quantitative methods are the specificity and applicability.

206. Reference Materials: According to ILSI 2001, and ISO Guides 30 to 35, reference materials play a number of roles in development, validation and troubleshooting of PCR-based diagnostics, as well as in the routine conduct of such assays. In the context of assay validation, positive reference materials are used to establish the accuracy, precision, sensitivity, LOD, and the false negative rate in quantitative assays. Negative reference materials are very important in determining the false positive rates and specificity. At the ISO guidelines on reference materials, it is defined that they can be of several levels of metrological quality:

207. A Certified or Standard Reference Material (CRM or SRM) is accompanied by a specific certificate. This certificate states that one or more of the property values of the reference material is certified by a procedure that establishes the value's traceability to an accurate realization of the unit in which the property value is expressed; in addition, the certificate states a level of confidence of uncertainty. Usually these reference materials are issued by National Metrology Institutes.

208. A Reference Material (RM) is a reference material or substance one or more of whose properties are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. (ISO definition).

209. A Working Standard (WS) is a secondary standard in regular use. This working standard is equivalent to RM if it is quantified/characterized by comparison with the CRM/SRM. In order to guarantee the quality standards for the reference materials, each one should be accompanied by a certificate of analysis. The certificate will describe the characteristics of the material, both as to the presence of the target material, and the absence of other possibly interfering materials. In addition, a reference material may even be restricted as to the method for which it can be used or is validated. Usually, the certificate of analysis for a GM reference material will address the target event or sequence; adventitious presence of other events; strength and purity; and genetic background. The references consulted call the attention for the choice of the reference material, as they can have advantages and disadvantages, according to particular purposes and usually there are three reference materials being used: Grain or seed or seed-derived powders; GM DNA of plant origin; Plasmid DNA or amplicons containing the target sequence. As I am not an expert in detection, I asked the EMBRAPA technicians in this area and they pointed out that the key decision is the type of reference material to use. This decision will be influenced by the availability of reference materials and must consider the matrix effects. In any case, each method should be validated in the laboratory using a reference material of the highest metrological standard available (SRM or CRM if possible). After the validation, the laboratory may use a reference material or working standard that has been calibrated back to the CRM/SRM.<sup>42</sup>

210. Answer 8c: Qualitative methods indicate the presence or absence of the analytical target; quantitative methods give a measure of the quantity of the analytical target present. For the purpose of verifying compliance with thresholds, both qualitative and quantitative DNA and protein based methods can be used. It is essential to establish appropriate sampling plans for both methods. Statistical models that build the foundation of the analysis of grain and seeds are made available through ISTA and GIPSA/USDA.

211. An event-specific DNA-based method determines the presence of a specific trait at a specific locus in a plant. A construct specific DNA-based method determines the presence of a gene within a

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<sup>42</sup> References:

International Standardization Organization, Geneva, Switzerland:

ISO Guide 30:1992 "Terms and definitions used in connection with reference materials",

ISO Guide 31:2000 "Reference materials -- Contents of certificates and labels",

ISO Guide 32:1997 "Calibration in analytical chemistry and use of certified reference materials",

ISO Guide 33:2000 "Uses of certified reference materials",

ISO Guide 34:2000 "General requirements for the competence of reference material producers",

ISO Guide 35:1989 "Certification of reference materials -- General and statistical principles".

ILSI (2001) – ILSI Europe Report Series: Method Development to in Relation to Regulatory Requirements for the detection of GMO's in the food chain. Summary report of a joint workshop held in December 2000. March 2001 (Prepared by the ILSI Europe Novel Food Task Force in collaboration with the European Commission's Joint Research Centre (JRC) and ILSI International Food Biotechnology Committee)

plant, independent of its specific integration locus; a screening method determines the presence of certain genetic elements, such as a promoter or a terminator. Protein based methods confirm the absence or presence of specific expressed traits such as herbicide tolerance or insect protection proteins.

212. Detection methods as applied to food or feed labeling do not have a direct role in risk assessment or risk management. However, while similar methods are used to establish the level of proteins in genetically modified products, and these levels are relevant to dietary intake and exposure assessments that form the basis of risk assessment, the common understanding of "detection methods" is that they are used to describe the presence or absence of GMO grain (or seed) in the context of conventional grain (or seed). Thus, there are two kinds of methods: methods that measure the analyte in the grain for the purposes of characterization and risk assessment, and methods that detect the presence or absence of the GMO (or level of the GMO) in seed, in grain or in a finished food or feed. The manuscript referred to above describes the use of detection methods for this second purpose.<sup>43</sup>

**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?**

**Dr Nutti**

213. Answer 9: According to the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003, Paragraph 18), the food safety assessment follows a stepwise process of addressing relevant factors. Molecular characterization informs the risk assessment the steps of the food safety assessment related to the description of the recombinant-DNA plant, description of the genetic modification and characterization of the genetic modification. However, all the safety assessment steps must be fulfilled. My understanding is that the knowledge achieved with a comprehensive molecular characterization of a transformation event is fundamental for the food safety assessment of a biotech product and, consequently, for its risk assessment.

214. The molecular characterization of a particular biotech product can be used to determine the presence and absence of certain DNA-sequences. It can be further used to determine the integrity of the inserted DNA, whether the sequence that was intended to be introduced was indeed introduced and whether it was introduced in a way that produces the expected protein products. Specific types of molecular characterization could also be used to assess the amount of a certain DNA-sequence.

215. Sometimes, the term molecular characterization includes the biochemical characterization of expressed proteins, and will include the description of the mode of action of a protein. Thus, conclusions about the safety of an inserted DNA sequence and its expression product is dependent upon: potential effects resulting from the assessment of the function of a gene, the mode of action of any encoded proteins; the relatedness of the inserted protein to proteins that have a history of safe use or are related to protein toxins or allergens. This evaluation needs to incorporate the biochemistry of

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<sup>43</sup> References:

ILSI (2001) – Ilsi Europe Report Series: Method Development to in Relation to Regulatory Requirements for the detection of GMO's in the food chain. Summary report of a joint workshop held in December 2000. March 2001 (Prepared by the ILSI Europe Novel Food Task Force in collaboration with the European Commission's Joint Research Centre (JRC) and ILSI International Food Biotechnology Committee)

the organism the gene was introduced into, the organisms' intentional use, the conditions under which the organism is cultivated, its interactions with the environment, and related factors.

**Dr. Andow**

216. A risk assessment cannot be carried out in the absence of a comprehensive molecular characterization of each transformation event. A risk assessment could not address the risks specific to the transgene of interest without this information. Without the information, there would be no clear basis for knowing when all reasonable scientific risks have been assessed and there would often be an uncertain basis for determining if a specific risk was scientifically justifiable or unjustifiable.

217. Molecular characterization addresses three distinct issues: (1) specification of expected expression of the transgene in the plant; (2) characterization of expression of the transgene in the plant; (3) genetic stability of the transgene under transmission to offspring.

218. Specification of the expected expression of the transgene in the plant is necessary to characterize expression and to begin to clarify scientifically what can be considered an expected effect and an unanticipated effect. Since about the year 2000, it has been technically routine and reliable to determine the number of transgene loci, the copy number at each locus, and of DNA sequence each transgene locus. For some crops, most notably maize, it was also possible to sequence out from the transgene through adjacent DNA until distinct plant DNA sequences could be identified (these are the so-called flanking regions). In 2001 this was not possible for some crops, such as cotton. Many other molecular methods are under development, including proteomics and others, but these are not yet ready for use in risk assessment now. It is likely that some of these tools will become available in the future.<sup>44</sup>

219. Characterization of expression of the transgene in the plant is necessary to specify all gene products from the transgene locus or loci and to determine their patterns of expression (concentrations of gene products) in the plant, both as the plant develops over time and as it generates new tissues and plant parts. All gene products must be identified so that their effects can be assessed. If they are not all identified, unanticipated effects could result from the unidentified products. It is important to characterize patterns of expression because transgenes are typically expressed differently in different plant tissues and expression in a plant tissue typically changes over time. In several cases, measured differences among tissues and changes over time have proven significant for risk assessment.

220. Genetic stability of the transgene under transmission to offspring is needed to demonstrate the transgene behaves similarly to other plant genes through development and reproduction. If it was not stably inherited, most likely it would be silenced or excised or otherwise inactivated. In some such cases, this could lead to unanticipated effects.

221. There is a paper that outlines the rationale for molecular characterization in detail for a particular case.<sup>45</sup>

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<sup>44</sup> Nap, J.-P., A. Atanassov and W. J. Stiekema (eds). 2004. *Genomics for Biosafety in Plant Biotechnology*. IOS Press: Amsterdam, 247 + viii pp.

<sup>45</sup> Andow, D. A., D.A. Somers, N. Amugune, F. J. L. Aragão, K. Ghosh, S. Gudu, E. Magiri, W. J. Moar, S. Njihia and E. Osir. 2004. Transgene locus structure and expression of Bt maize, pp. 83-116. In A. Hilbeck and D.A. Andow (eds), *Environmental Risk Assessment of Transgenic Organisms: A Case Study of Bt Maize in Kenya*. CABI: Wallingford, UK.

**Dr. Healy**

222. **General Approach to Assessing Safety:** It is well accepted that assessment of whole foods, such as foods from genetically modified organisms (GMOs; GM foods), involves the use of the 'comparative approach', whereby GM foods are compared to their counterpart foods produced using conventional techniques and which have been safely consumed over significant periods of time. The objective of such a comparison is to identify any differences with the conventionally produced food. Any identified differences are then examined for their biological impact in terms of health and safety.

223. This approach to the assessment of the safety of food products has been advocated for over a decade (OECD, 1993,1996; WHO, 1991; 1996) and reconfirmed several times during that period. Most notably, a relatively recent expert consultation convened by WHO/FAO concluded that 'a comparative approach.....is considered the most appropriate strategy for the safety and nutritional assessment of genetically modified foods' (WHO, 2000). This approach is used in place of the traditional approach of assessing the safety of discrete chemicals added to, or present in, food (eg food additives, contaminants) that are generally subjected to a range of toxicological evaluations in animal studies in a dose dependent manner (WHO, 1987). The toxicological approach is not appropriate for whole foods because of the complex mixture of chemicals present in a whole food, some of which will be present at very low levels, and the difficulty of conducting meaningful dose response studies in animals while providing a nutritionally balanced diet.

224. The comparative approach to examining the safety of a GM food is generally considered to encompass three major elements: molecular characterisation of the inserted gene (transgene); biochemical, structural and functional properties of the protein product from the inserted gene; and analysis of the composition of the food. This approach taking into account knowledge about the organism from which the transgene is sourced as well as the recipient organism. Application of the comparative approach has been examined in detail for food derived from GM plants (WHO, 2000; WHO, 2001a), GM micro-organisms (WHO, 2001b) and GM animals (WHO, 2003).

225. The totality of information available from these types of studies should be considered in coming to a view about the safety of the food; information from each element contributes to the assessment of safety. Analyses of the information about these three aspects of the GM food provides a structured mechanism to identify both the intended changes to the food as well as any that may be an unintended consequence of the genetic manipulation. Some unintended consequences may be predicted while others are unexpected.

226. The comparative approach involving characterisation at the DNA, protein and compositional levels has been generally accepted and applied by many countries around the world, some for more than a decade. These countries include Australia and New Zealand (ANZFA, 2001), Belgium (Van Haver et al, 2003 ), Canada (Health Canada, 1994), European Union Member States (SCP, 1999; EFSA, 2004), Japan (Japanese MHLW 2001), United Kingdom (UK FSA, 2003) and the United States (USA FDA, 1992, 1997, 2001). Approaches by other countries are summarised in OECD, 2000 and Kuiper et al, 2001. A significant number of foods from GMOs have now been approved using this assessment approach (eg Japan<sup>46</sup>, Australia/New Zealand<sup>47</sup>, Canada<sup>48</sup>, USA<sup>49</sup> and Europe<sup>50</sup>). International consensus on the appropriateness and utility of this approach resulted in the development

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<sup>46</sup> <http://www.mhlw.go.jp/english/topics/qa/dna/index.html>.

<sup>47</sup> <http://www.foodstandards.gov.au/whatsinfo/gmfoods>.

<sup>48</sup> <http://www.he-sc.gc.ca/food>.

<sup>49</sup> <http://www.cfsan.fda.gov/~lrd/biocon.html>.

<sup>50</sup> [http://europa.eu.int/comm/food/food/biotechnology/authorisation/258-97-ec\\_authorized\\_en.pdf](http://europa.eu.int/comm/food/food/biotechnology/authorisation/258-97-ec_authorized_en.pdf).

of international guidance on the assessment procedures by the body responsible for developing international food standards, the Codex Alimentarius Commission (CAC, 2003a, b, c, d).

*Role of Molecular Characterisation in Risk Assessments*

NOTE: QUESTION 9 REFERS TO THE ROLE OF MOLECULAR CHARACTERISATION IN A 'RISK' ASSESSMENT. HOWEVER, I HAVE ANSWERED THE QUESTION IN THE CONTEXT OF A 'SAFETY' ASSESSMENT, WHICH IN RELATION TO EXAMINING THE HEALTH AND SAFETY IMPACT OF FOOD, HAS A DEFINED MEANING (CAC 2003A) AND MAY BE PART OF A SUBSEQUENT RISK ASSESSMENT IF POTENTIAL HAZARDS ARE IDENTIFIED DURING THE SAFETY ASSESSMENT.

227. The molecular characterisation is considered to be a very important part of the safety assessment of a biotech product. It provides information about the number of copies of the transgene(s) that have been inserted, presence of any multiple copies at the same or dispersed insertion site(s) and the structural organisation of the inserted transgene(s). The molecular characterisation also includes an analysis of the stability of the inserted DNA. Together this information provides verification that the intended genetic modification has occurred and provides some indication as to the likelihood of any unintended changes. However, the molecular characterisation is not the only mechanism for identifying unintended effects and the information from such studies should be considered in conjunction with the information from the other elements of the safety assessment.

228. While a safety assessment could be conducted in the absence of a comprehensive molecular characterisation, current international good practice includes the molecular characterisation (CAC b, d). However, the issue does arise as to how comprehensive the molecular characterisation should be in order to fulfil the requirements of an appropriate safety assessment. In large part, the answer to this question depends on the type and quality of information available for the three elements of the safety assessment, the information revealed from these studies and the state of the scientific knowledge about the gene(s) to be transferred, the donor organism and the recipient organism.

229. In recent years, there have been significant developments in the technologies to analyse the molecular biological and biochemical characteristics of GMOs and their products (eg DNA sequence analysis, reverse transcriptase polymerase chain reaction, bioinformatics; micro array technologies, proteomics and metabolomics). In some cases, these technologies can already significantly assist the safety assessment. A recent example is the ready generation of DNA sequence data. It should be noted that the guidelines for conducting safety assessments of GM foods that were recently adopted by the Codex Alimentarius Commission (CAC 2003 b, d) require information sufficient to identify any new substances that may be included in food. DNA sequence information on the junction regions of the transgene and for the transgene itself is one mechanism to obtain this information.

230. It is now regarded as good practice to undertake DNA sequence analysis as part of a safety assessment, for several reasons. First, some of the gene constructs and the transformation technologies available today result in more complex segments of DNA and in rearrangements of the inserted DNA. One of the most effective methods currently available for assessing the integrity and impact of the inserted DNA is sequencing. Second, sequence information can now be much more readily generated than was possible using the laborious procedures available a decade ago.

231. Although this type information has not generally been required in the past, a number of countries have now included requirements for its provision in the future. However, countries have

generally not required such information for GMOs and their food products that have previously been approved for entry to the marketplace.

232. Some of the technologies being developed are currently not appropriate for use in safety assessments as they do not yet provide any further assurance of safety or the results cannot be fully interpreted (eg NAS, 2004). However, some of the profiling methods, such as metabolomics, may eventually prove very useful. At some point in the future, the development of these technologies and associated understanding about the impact of small variations in gene expression levels and/or metabolites may be adequate, minimising the need for molecular characterisation.

233. It must also be acknowledged that the commercially produced GMOs generated to date have utilised well defined crops as recipients of the genetic modification and well defined genetic elements in the transgenes. Generally, the genetic elements have been extensively studied at both the DNA and protein levels. Consequently there has been a high level of confidence in the assessments carried out to date. However, there have been ongoing developments in the molecular biological technologies that are being applied in the development of GM crops (eg different transformation technologies; insertion of multiple and different genes; different donor and recipient organisms) and these are raising new and more complex questions about the safety of the products of such crops in the food supply.

234. *Conclusion:* Currently the molecular characterisation is an important element of the safety assessment, providing verification that the intended genetic modification has occurred and information about unintended effects. The extent of molecular characterisation in any particular case should be addressed in the context of the available scientific information.

235. **Approach to Addressing Specific Questions About Molecular Characterisation:** Questions relating to requests for additional information for safety assessment purposes have been considered in the context of the general approach to assessing the safety GMOs and their food products. In particular, requests for further molecular characterisation have been considered according to the principles and approaches outlined above.

#### **Dr. Squire**

236. The Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants indicate full characterisation of the genetic change should be provided. Such information may be essential for assessing some aspects of the product but is not always necessary when considering spread or persistence of the biotech product or its effect on the ecosystem.

237. Spread and persistence. In many of the studies in Europe (ongoing and not yet published), the purposes of the study can be achieved by detection based on an outward or phenotypic trait (i.e. whether a plant dies if sprayed with a specific herbicide), a protein test (e.g. an antibody based test for the protein produced by the genes conferring tolerance to glyphosate or glufosinate ammonium) or a DNA test for the gene (e.g. the bar gene). None of these tests is absolutely accurate since they are affected by the condition of the plant tissue. If sampling of plant material is done in real agricultural fields, it is impossible to ensure the plant tissue is in the right condition – often it is not. Consequently, false-positives and false-negatives occur. Moreover, if the GM trait is thought to be at low frequency, e.g. 0.5% or lower, and the individuals possessing it are unevenly distributed or clumped in the field, then very large numbers of individuals have to be sampled to estimate presence or % frequency with a high degree of certainty.

238. Ecological impact. 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.

239. More information would be necessary if research was following the volunteers or pollen-vectored genes originating from the GM crop: at least, knowledge of the genetic factors determining the proportions of male sterility and male fertility would be important, but even then the detailed molecular knowledge of the construct would not be essential. However, if many GM varieties had been grown in an agricultural area, and if it were important to ascertain the origin of an individual plant or population, then more detailed molecular characterisation would be required.

240. More generally it can be argued, given the scientific and public interest in GM issues, that it is reasonable for an assessing body to request full characterisation (according to standards in the Guideline for the conduct...) of a GM construct (a) to enable its officers to confirm whether impurities existed in the original seed if they were found or suspected (e.g. the presence in the breeding line of GM traits such as antibiotic resistance) and (b) to trace the persistence or spread of the GM trait if this was necessary in later years.

241. While much of the text above under Responses 9 is generally appreciated by workers in the field, many of the scientific findings pertaining to the spread and persistence of GM traits in Europe are not yet in the public domain (Notes, paragraph 14).

## **ISSUE 1**

*Background: The following questions have the objective of assisting the Panel to determine whether the EC regulatory approval process for the specific biotech products at issue in this dispute proceeded without unjustified delays on the part of the relevant EC regulatory bodies. The questions are aimed at determining for each product application (whether pending or withdrawn):*

*(a) the scientific or technical grounds for the comments and/or objections raised by EC member States following the initial assessments of the Competent Authority (CA) and of the relevant EC scientific body, in light of the scientific evidence and evaluations available at the time;*

*(b) the scientific or technical grounds for the requests for additional information from the notifier during or following the initial assessments of the CA and of the relevant EC scientific body, in light of the scientific evidence and evaluations available at the time; and*

*(c) the justification on scientific or technical grounds for the time taken to evaluate the additional information provided.*

## **Questions:**

**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 Scientific Committee for Plant (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?**

**Dr. Nutti**

242. Based on the information provided by the applicant and the analysis carried out by the EC Scientific Committee for Plant (SCP) and following the Codex Guidelines, my understanding is that the request by the Italian CA (EC-62/At.95) was not necessary to ensure that conclusions for the safety assessment of the newly expressed protein in the food chain were valid. As pointed out by the EC SCP, the new protein has a very low potential for being allergenic or toxic and also is degraded within the gastric fluids. In my opinion, the conclusion of the EC SCP that the protein was safe for consumption in the food chain is correct and was based on sound scientific evidence, which was presented by the applicant.

**Dr. Andow**

243. The request by the Italian CA (EC-62/At.95, 14 March 2000) is repeated below.

Referring to the conclusion of the last meeting of the Regulatory Committee under Directive 90/220/EEC, Italy would be very pleased to receive from the notifier more information concerning the assessment of the effect of the genic product on the biogeochemical cycles and on food chain and on the apreading of the gene due to the possibility of crossing between the PGM and wild species.

244. The original notification by AgrEvo (EC-62/At.1-30, 1 April 1996) contains no studies on the safety of Falcon GS 40/90 to other organisms and no studies on outcrossing rates with wild species. Instead, the notifier argues that Falcon GS 40/90 is in no way different from non-transgenic cultivars expect for the ppt gene. They conclude that there is no reason to believe that Falcon GS 40/90 has any adverse effects on the environment.

245. In my reading of the original notification, AgrEvo's claim rests entirely on the SCI report "Glufosinate tolerant transgenic Winter Oil Seed Rape – Field observations of the variety Falcon GS 40/90 pHoe6/Ac in comparison to Falcon non transgenic and conventional farmers rape," which is code number Hoe 039866 00 SL 01 A 201 (EC-62/At.30\_SCI, 1 April 1996), and on the report "Supplementary ecological monitoring of a transgenic, Basta-resistant winter rapeseed crop cultivated at Friemar (Thuringia, Federal Republic of Germany) during the season 1994/95" (EC-62/At.8). I agree with the assessment of the lead CA that EC-62/At.8 is difficult to interpret. Without going into the details, the data are not scientifically convincing that the environmental effects of Falcon GS 40/90 are in no way different from non-transgenic cultivars.

246. The lead CA requested improvements to EC-62/At.8 on 14 May 1996 (EC-62/At.33) and received an improved version from the notifier on 12 July 1996 (EC-62/At.37\_SCI). This includes a revision of EC-62/At.8. The notifier admits that the small plot size in EC-62/At.8 precludes statistical analysis, so I conclude that the data are of limited scientific value for evaluating environmental effects.

247. The full application to the community was not included in documents before the Panel, so it is not possible to determine conclusively the basis for the SCP assessment.

248. Between the time of submission of the full application to the community and the time of the SCP Opinion, there were several requests for information to the notifier from various countries and

the SCP. The replies from the notifier are in EC-62/At.45, EC-62/At.52, EC-62/At.54, EC-62/At.68 and EC-62/At.71. Information in EC-62/At.52 and EC-62/At.71 are relevant to the question at hand.

249. In EC-62/At.52, 28 January 1997, the notifier clarified as follows:

**42. Survival, multiplication, and dissemination of the GMO(s) in the environment:**

The comparison study mentioned in this section has only taken place in a growth chamber which is big enough to handle even maize plants. Field observations are discussed in document A 56991.

Five plants each from the recipient and from the transformant have been analysed in the growth chamber.

250. In EC-62/At.71, 5 June 1998, the notifier provided extensive information related to environmental impact that was not available previously. This addressed dispersal of oilseed rape pollen, the potential invasiveness<sup>51</sup> of Falcon GS 40/90 oilseed rape, and a number of aspects related to fitness, weediness and outcrossing (data and sources not provided).

251. The SCP concluded that there is no evidence to indicate that Falcon GS 40/90 is likely to cause adverse effect on human health and on the environment (EC-62/At.74, 14 July 1998). However, they also indicate that "Few studies have been conducted on the safety of modified oilseed rape to other organisms." No studies were conducted on biogeochemical cycling or on the food chain.

252. It must be stated clearly at this point that the lack of evidence does not imply that there is a lack of an effect. Either the SCP did not base their conclusion about non-target effects on sound scientific reasoning or the reasoning they used is not apparent from their Opinion (EC-62/At.74). In either event, their conclusion does not follow from the data provided by the notifier.

253. The SCP did address the possibility of the spreading of the gene due to crossing between Falcon GS 40/90 and wild species. The SCP indicated that the likelihood of crossing to wild species was small, especially when compared to the likelihood of crossing with another oilseed rape cultivar. They suggested that appropriate risk management measures would be likely to avoid these risks.

254. The conclusions by the SCP related to outcrossing and invasiveness are based on the sound interpretation of the data present at the time. However, there is likely to be several approaches to risk management, and these are not specified.

255. The Italian CA (EC-62/At.95) requested information to assess the long-term effect of the newly expressed protein on the biogeochemical cycle and the food chain. While a request for additional/ clearer assessment of the possible effects on environment can be readily justified on the basis of the preceding analysis, such a request must itself be clear and specific. For example, it is not clear that all of the environmental hazards have been identified let alone assessed. A request for additional/ clearer assessment of the possible effects on the environment would not be clear or specific enough to allow a notifier to know how to respond, and hence although information on the environmental impacts is necessary to ensure that conclusions of the safety assessment were valid, a general request for such an assessment would not be an appropriate way to request such information. In a similar way the request by the Italian CA is not clear or specific enough to be considered

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<sup>51</sup> Crawley et al. Nature 363: 620-622.

necessary. There are hundreds of possible processes in biogeochemical cycles that could be investigated, and thousands of possible ways to evaluate food chains. Unless the Italian CA can point to prior regulatory precedents where these terms are clarified, the phrasing of the request is not scientifically justified. Alternatively, had the Italian CA specified certain biogeochemical cycles and food chains to assess, then it would be possible both for the notifier to assess them and for me to determine their necessity to ensure that conclusions of the safety assessment were valid.

256. The Italian CA request came on 14 March 2000. On 10 November 1999 Aventis submitted and updated notification, which was circulated to the CAs on 13 December 1999. The AFFSA Opinion was issued 5 January 2000 and Aventis provided additional information on 28 February 2000. The time between the receipt of materials until the Italian CA response can be calculated as either 15 or 125 days. Of the 125 days, 33 were used to process the file administratively, and Italy had the materials for 92 days. The shorter time is a very rapid response time. The longer time is probably too long, but mainly because 33 days seems excessive for processing the file administratively.

**Question 11: Given the information before the Panel, including the notification by AgrEvo and the conclusions of the EC SCP, was the information regarding the post-event monitoring protocol to assess the likelihood of spreading requested by the lead CA necessary to ensure that conclusions of the safety assessment were valid (EC-62/At.100)? If so, what other risk management options are available to mitigate this risk?**

**Dr. Nutti**

257. Three questions presented in EC-62/At.100 are on environmental issues. I can only refer to item 7.3 (oilseed rape animal feeding study), where a doubt was raised about which event was used in the male broiler chicken studies. It is mentioned in EC-62/At.103 that copies of both studies were submitted by the notifier in order to answer this point. I could not find these studies in the attachments, but examining the information provided by the applicant to EC and the answers given in EC 62 (Attachments 003, 005, 006, 007, 010, 011, 012, 015, 017, 018, 019, 021, 022, 023, 024, 025, 026, 027, 028, 049, 050, 051, 053, 057, 060, 063, 065, 071, 073, 074, 075, 081, 082, 085, 091, 093 and 0103) and the analysis carried out by the EC Scientific Committee for Plant (SCP) (Exhibit US-42), where it is relevant to note:

258. Item 7 (Overall Assessment): "there is no evidence to indicate that the placing on the market of line transformant Falcon GS 40/90 oilseed rape, with the purpose to be used as any other oilseed rape is likely to cause adverse effects on human health and on the environment".

259. Item 6.2.2: "in food of animal origin from livestock animals fed with feedingstuffs after the application of glufosinate-ammonium to tolerant rape, no residues are expected above the limit of determination".

260. My understanding is that there is no significant risk to humans or livestock with the ingestion of the gene product. All the food safety aspects indicated in the Codex Alimentarius Guidelines have been accomplished. It is important to point out that the nutritional composition of the entire seed, meal and oil from the genetically modified rape has been analysed. Information on the contents of oil, fibre, ash, protein, fatty acid composition, glucosinolate, sterols and tocopherols showed no differences that could be attributed to the genetic modification. The Scientific Committee for Plants appointed within the EC Commission has, in its opinion on rape, stated that no significant changes in nutritional value have been identified.

261. I cannot express any opinion on the post-event monitoring protocol since this is an environmental issue. According to the follow-up programme, monitoring will be carried out on herbicide use, biodiversity and spread of the genes introduced via waste plants and wild relatives.

**Dr. Andow**

262. The information requested by the lead CA regarding the post-event monitoring protocol to assess the likelihood of spreading is in EC-62/At.100, 25 July 2001, and reproduced below.

Ad\_Document\_3 ("Draft Post-Marketing Monitoring Plan for InVigor®, SeedLink® and LibertyLink® Oilseed Rape"):

*III.2. "Existing networks of expertise to carry out General Surveillance":*

On principle, we think that a more detailed description of the tasks and the kind of data that will be collected by the different networks would further acceptance of the monitoring plan by the other Member States. We expect that relevant information will be supplied by Aventis after the different networks have elaborated their programs for the general surveillance.

*III.2.2. "Plant protection networks":*

In Germany, the "Bundessortenamt" is the competent authority for plant variety registrations (as mentioned under III.2.1.), but its focus is not on evaluating the impact of agricultural practices on the environment, specifically on water, soil, wildlife and flora. Since several federal and regional ("Länder") authorities are responsible for these questions in Germany, it would be difficult to give a complete list. We suggest either to omit reference to a particular institution in Germany or, alternatively, to mention – as the main federal and regional institutions – the "Biologische Bundesanstalt für Land- und Forstwirtschaft", the "Umweltbundesamt" and the "Pflanzenschutzämter".

263. The first mention of a monitoring protocol is in the SCP Opinion (EC-62/At.74, 14 July 1998).

2. The SCP is also of the opinion that the potential transfer of the herbicide resistance gene to wild Brassica relatives is a new issue in Europe in view of the limited scale of release to date. The SCP has examined the available evidence from monitoring and research programmes to date. After evaluating all the information available to the SCP, it was concluded that herbicide tolerant volunteers that may appear would be canola plants and not wild Brassica relatives. Such herbicide-tolerant volunteers could be controlled in subsequent crops by conventional agricultural methods other than by the use of glufosinate-ammonium. The SCP recommends that the introduction of herbicide tolerant crops should be accompanied by:

- i) an agreed code of practice for field management of the particular modified crop involving the active participation of the notifier to promote best practice by farmers.
- ii) a research 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.

264. The SCP is recommending a "code of practice" in order to ensure that herbicide-tolerant volunteers are eliminated. In other words, the purpose of the risk management measure is to eliminate volunteers as they occur. In addition, it is recommending research design and implement monitoring to detect herbicide-tolerant volunteers and herbicide-tolerant weeds in the EU.

265. The notifier indicates they are developing a monitoring plan in EC-62/At.75, 22 June 1998, even before the SCP is published), with some details in Annex 3. Annex 3 is not a part of the materials before the Panel.

4. that PGS and AgrEvo contribute to and collaborate with different programmes monitoring the occurrence and establishment of herbicide tolerant volunteers and weeds under field conditions. In annex we present examples of collaborations in the UK, France and Germany. PGS and AgrEvo will keep the Scientific Committee on Plants as well as the competent authorities informed of the results.

266. The notifier provided a monitoring plan (EC62/At.78, 25 January 1999) entitled "A Structured Stewardship and Monitoring Programme for Agronomic Impact of Growing LibertyLink™ and SeedLink™ Oilseed Rape in Europe – A monitoring and quality assurance strategy." The main objective of this plan is to increase understanding of the appropriate management of these products by their users and to improve public awareness and acceptance.<sup>52</sup> The second objective is to promote practices, which ensure the quality and stability of the crops, in both the farmers' and AgrEvo's own interest.<sup>53</sup>

267. In the monitoring plan is a section entitled "2.3.2 Which Guidance?" in which the notifier states that they will provide farmers with guidance on volunteer management: post-harvest treatment of the area. However, there are no details on the guidance that will be provided. Moreover, there is no concrete discussion on how a monitoring plan will be implemented (see my answer to question 24a for criteria on judging the completeness of a monitoring plan).

268. This monitoring plan (EC62/At.78, 25 January 1999) does not meet the specifications suggested by the SCP.

269. In EC-62/At.81-82, 8 July 1999, the notifier repeats information in EC-62/At.71, 5 June 1998.

270. In EC-62/At.83, 3 August 1999, the notifier reiterates the content of EC-62/At.78, 25 January 1999.

271. In EC-62/At.90\_SCI, 10 November 1999, the notifier modifies the proposed monitoring plan from that in EC62/At.78, 25 January 1999. The main objective is still to increase understanding of the appropriate management of these products by their users and to improve public awareness and acceptance. There is no secondary objective. [xxx]

272. The lead CA requested (III.2 in EC-62/At.100, 25 July 2001) more detailed description of the tasks and the kind of data that will be collected by the different networks. As the monitoring objective of the notifier does not fully correspond with the monitoring recommendations of the SCP, or monitoring objectives of what will become Annex VII of Directive 2001/18/EC, and there are few details in any of the proposed monitoring plans submitted by the notifier, and the submitted monitoring plans do not address the main management measure recommended by the SCP (how

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<sup>52</sup> EC-62/At.78, p. 9 of pdf file.

<sup>53</sup> EC-62/At.78, p. 9 of pdf file.

conventional practices will eliminate volunteers), this request for additional information is necessary to ensure that conclusions of the safety assessment were valid.

273. The lead CA also requested (III.2.2 in EC-62/At.100, 25 July 2001) a modification to the list of proposed plant protection networks that will participate in monitoring. This seems like a useful and important suggestion to the notifier, and should be considered necessary to ensure that conclusions of the safety assessment were valid.

274. There are many other possible risk management options to the monitoring systems proposed by the notifier. The most evident is to follow up rigorously on the SCPs original suggestion that conventional practices may be sufficient to eliminate volunteers. This would require the notifier to consider all the possible practices that may follow an oilseed rape crop and determine which aspects of these are responsible for elimination of volunteers. I would suggest that the competitiveness of the subsequent crop, the phenology of crop management activities (disturbance regime), and management of field margins (tillage or herbicide application) may be critical factors that would be sufficient to eliminate nearly all volunteers. Monitoring would shift from looking for glufosinate-tolerant volunteers and weeds to monitoring the cropping practices of farmers. Monitoring for glufosinate-tolerant volunteers and weeds could then be restricted to those farmers or regions where management of the subsequent crop was inappropriate for eliminating volunteers.

275. The lead Ca requested the information on 25 July 2001. The notifier provided the reviewed proposal on 10 November 1999. This is a 1 year 8 month interval, and is excessively long.

#### **Dr. Snow**

276. In summary, asking for information about the post-event monitoring protocol appears to be justified for two reasons. First, when the EC SCP rendered its opinion in July 1998, questions were raised about how quickly transgenic resistance to glufosinate would spread to volunteer oilseed rape plants and to weedy Brassica rapa. The EC SCP stated that these questions could be addressed by post-release monitoring. Second, information about monitoring is now required under EC Directive 2001/18, which went into effect in March 2001. Discussions about whether to require monitoring protocols were probably taking place while the previous Directive was in effect as well.

277. With regard risk management options, the spread of volunteer oilseed rape or wild Brassica species that acquire resistance to glufosinate can be managed by other methods of weed control, including applications of other herbicides. However, if glyphosate-tolerant oilseed rape is introduced in the future, problems with herbicide-resistant volunteers could be compounded. This concern is mentioned specifically in the EC SCP (EC-062-At. 074).

278. I noticed two shortcomings in the notification for Falcon GS40/90 by AgrEvo (EC-062-At. 003) and the July 1998 conclusions of the EC Scientific Committee on Plants (SCP; EC-062-At. 074):

- (a) The documents show that both AgrEvo and the EC SCP concluded that transgenes from oilseed rape are unlikely to spread to weedy Brassica rapa and multiply, despite the fact that several early studies showed that persistence of the transgenes is expected (Jeorgensen and Andersen 1994, Mikkelsen et al. 1996, Snow et al. 1998). The SCP stated ". . . there may be a very low level of natural crossing with related species particularly Brassica rapa and Brassica juncea under field conditions. . . The risk assessment assumes that transfer will occur at a very low level." This could be correct, depending on local conditions. However, the EC SCP then stated: "After evaluating all the information available to the SCP, it was concluded that herbicide

tolerant volunteers that may appear would be canola plants and not wild Brassica relatives." The basis for this conclusion is not clear (also, it doesn't make sense to refer to wild relatives as a type of volunteer).

- (b) AgrEvo and the EC SCP did not specifically address the issue of how the widespread adoption of glufosinate-tolerant canola would affect the efficiency of weed control, which has implications for biodiversity in farmland habitats (see above). This question had not been evaluated at the time and is still under investigation.

### **Dr. Squire**

279. If long term events or emergent properties are envisaged as a result of introducing a GM plant and practice (and this seems to be the case for oilseed rape in Europe), then it is legitimate to expect a rigorous post-event monitoring protocol to be integral to a pre-release risk assessment. Risk management options for this GMHT product are similar to the generic ones included in Responses 6d-e. When they are applied, the practices appear to work in keeping weedy oilseed rape to manageable levels, but the general level and type of management have not prevented it from becoming established throughout Europe as a weed.

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

### **Dr. Healy**

280. In 1996 AgrEvo submitted a request to the German CA for the authorisation of the marketing of oilseed rape carrying the Falcon 40/90 transformation event. The German CA expressed a favourable opinion in late 1996. Subsequently, the request was forwarded to the European Commission and distributed to the Member States in December 1996. A number of the Member States raised concerns and requested that additional information be provided. The original dossier, together with additional material supplied, was considered by the Scientific Committee for Plants (SCP), with a favourable opinion being issued in mid 1998.

281. Information Submitted (EC 62 – 1-30 37, 73): The recipient oilseed rape line was transformed with a gene encoding the enzyme phosphinotricin acetyl transferase (PAT) from a common soil bacterium. The PAT enzyme inactivates the herbicide glufosinate ammonium and confers herbicide tolerance to the genetically modified oilseed rape. The gene was constructed so that the regulatory sequences for the pat coding region were derived from the cauliflower mosaic virus. These genetic elements are well described in the literature and have been used to transform a variety of crops, both in the combination used in this case as well as in combination with other coding regions and/or regulatory sequences. Furthermore, a number of oil seed rape lines transformed with the pat gene, sometimes in combination with other genes, have been assessed for safety and approved for food use in a number of countries around the world (eg Japan<sup>54</sup>, Australia/New Zealand<sup>55</sup>, Canada<sup>56</sup>, USA<sup>57</sup> and Europe<sup>58</sup>).

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<sup>54</sup> <http://www.mhlw.go.jp/english/topics/qa/dna/index.html>.

<sup>55</sup> <http://www.foodstandards.gov.au/whatsinfood/gmfoods>.

<sup>56</sup> <http://www.hc-sc.gc.ca/food>.

<sup>57</sup> <http://www.cfsan.fda.gov/~lrd/biocon.html>.

282. 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. Analysis of the biochemical and structural properties of the protein included heat stability, pH optimum, enzyme kinetics, inactivation studies in the presence of gastric juices from a variety of species and under a variety of conditions. These studies were conducted with the PAT protein isolated from a variety of transformed organisms, including an over expressing E. coli strain and maize and oilseed rape line transformed with the pat gene, which were demonstrated to have similar immunological properties to each other. The DNA sequence of the pat gene used to generate Falcon GS 40/90 was compared to major sequence databases. These studies also allow an assessment of the allergenicity potential of the PAT protein.

283. The PAT protein was tested for dose responsive toxicity effects in animals. The treated animals were subjected to extensive analysis for toxic effects including mortality, food consumption, clinical parameters, clinical biochemistry and pathology on an array of internal organs.

284. The composition of fractions derived from seeds were analysed for nutrients appropriate for the food (oil) and feed derivatives of oilseed rape (OECD, 2001). The analysis addressed the key fatty acids in oil (including the toxin erucic acid) as well as nutrients and toxins in oilseed rape meal.

285. Conclusions of the Safety Assessment: The SCP in July 1998 (EC 62-74) concluded that having considered the submitted dossier and the background information available, 'there is no evidence to indicate that the placing on the market of line transformant Falcon GS40/90 oilseed rape, with the purpose to be used as any other oilseed rape, is likely to cause adverse effects on human health...'. Furthermore, the SCP did not request any additional information or suggest any additional research in relation to the safety assessment. It appears that the information submitted met the requirements for a safety assessment as outlined in Annex 11 B of Directive 90//220/EEC.

286. I have reviewed the scientific information submitted and consider that a reasonably comprehensive information package was developed to support the safety assessment of oilseed rape line Falcon GS 40/90. The information package developed was consistent with internationally accepted approaches to the safety assessment at that time, as outlined above. The information package addressed the three major elements that are usually considered in assessing the safety of a GM product and included molecular characterisation of the inserted gene, characterisation of the protein expressed by the inserted gene and an analysis of key compositional constituents relevant to the food product (ie oil) derived from the Falcon GS 40/90 line. The experimental procedures were conducted in accordance with standard methodologies.

287. Having examined the scientific information underpinning the safety assessment and the opinion of the SCP, I conclude that food (oil) derived from oilseed rape Falcon GS 40/90 line is as safe for human consumption as food from conventional produced oilseed rape varieties. There is no indication from the scientific evidence submitted for consideration by the Competent Authority that the food poses a health or safety risk. Furthermore, there is no indication of human health and safety issues arising from the consumption of food from other lines of oilseed rape carrying the pat gene that have been approved around the world and which are/have been in commercial production, some for significant periods of time. I therefore concur with the opinion of the SCP, issued in July 1998.

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<sup>58</sup> [http://europa.eu.int/comm/food/food/biotechnology/authorisation/258-97-ec\\_authorized\\_en.pdf](http://europa.eu.int/comm/food/food/biotechnology/authorisation/258-97-ec_authorized_en.pdf).

288. The Lead CA sought addition information (EC 62-106):

- on the DNA sequences flanking the transgene locus in Falcon GS 40/90 (ie spanning the left and right junctions between the inserted DNA and the plant DNA),
- comparison of these DNA sequences with those of the pre insertion site in nontransformed lines, preferably from the Falcon variety.

289. The information on the flanking sequences is likely to provide a full sequence analysis across DNA junction between the genomic DNA and the inserted DNA (pat gene). Such information can be used to analyse the DNA for potential novel proteins that may be generated as a result of the juxtapositioning of the different segments of DNA.

290. Such information will also provide a more detailed description of parts of the structure of the transgene that was actually inserted into the genomic DNA, facilitating the identification of any small rearrangements or other modifications to the DNA that may occur during the transformation process. Some transformation techniques are more prone than others to generating such modifications.

291. Comparison of the insertion sites in the transformed line with that of its parental non transformed line may give additional information about any minor DNA changes at these sites (eg rearrangements, deletions). It may also reveal other nucleotide changes (polymorphisms) that are a result of natural variation between individuals within lines and which are not related to the transformation process.

292. 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 rationale for this view are:

- 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, compared to some other transformation techniques (eg micro projectile bombardment). The molecular characterisation provided by AgrEvo does not indicate that any modifications to the structure of the inserted DNA have occurred although the possibility of small modifications that cannot be detected by Southern blot analysis cannot be entirely excluded. This is well exemplified by the example of Roundup Ready soybean, for which the initial molecular characterisation did not reveal any modifications in and around the inserted gene, although subsequent sequence analysis revealed the presence of additional DNA fragments (Windels et al, 2001).
- 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.
- Additional information of the sequences at the sites of insertion may reveal fine structural details and identify similarities and differences with the parental line at these sites. Such detailed structural analyses usually aim to provide an indicator of unintended effects from the genetic modification. However, in some cases it may be difficult to determine the biological significance of any sequence differences because of a lack of knowledge about the extent of sequence variation between individual organisms within the parental line. Furthermore, other indicators of unintended effects, that are biologically meaningful, are obtained from other elements of the safety assessment (eg analysis of protein, composition of food).

- A comprehensive package of information was provided that addressed the key elements required in a safety assessment and there was no indication of any safety issues when all the available data was taken into account. Furthermore, the pat/PAT gene/protein is well characterised, has been utilised successfully in a number of crops and their resultant food products and was derived from a well described organism.

293. Conclusion: 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. The totality of information provided in the dossier to that point in time were adequate to support the validity of the conclusions of the safety assessment without the need for the additional data requested in the letter dated 2 April 2002.

### **Dr. Snape**

294. The comments below relate to Question 12 concerning the molecular characterisation of exhibit EC-062. These were examined relative to the EFSA-Q-2003-005 guidance document produced by the Scientific Panel on Genetically Modified Organisms.

295. Exhibit EC-062: Oilseed rape event Falcon GS40/90 (also named GS40/90pHoe6/Ac) was produced, using plasmid pHoe6/Ac, by means of Agrobacterium-mediated gene transfer. The T-DNA of the plasmid pHoe6/Ac used for transformation carries a chimeric pat expression unit conferring resistance to the herbicide glufosinate. Oilseed rape event GS40/90pHoe6/Ac contains two functional transgenic loci. Both transgenic loci will be present in the commercialised GM plants. Information relative to the molecular characterisation of GS40/90pHoe6/Ac has been found primarily in EC-062 attachments 3, 13, 14, 113, 114. A series of comments and questions are numbered below. This is followed by a conclusion proposing answers to Question 12.

296. The molecular characterisation of GS40/90pHoe6/Ac initially provided was not complete and failed to identify the exact transgenic make-up of this GM plant. Initially, two intact copies of the CaMV35S:pat:35T expression unit were believed to be inserted at two independent functional loci (EC062 attachment 3 and 14). Later it was determined, following requests for additional information, that in fact one of the two functional loci contained two CaMV35S:pat:35T expression units in inverted tail-to-tail orientation (EC062 attachment 114) bringing the total copy number of the pat gene to three in GS40/90pHoe6/Ac. This re-emphasizes the need, expressed in general comment 2 (accompanying document) by this Expert, to conduct independent molecular analyses for each transgenic locus present in a GM plant. This also means that two sets of full molecular analyses are expected to be provided, one for each transgenic locus present in GS40/90pHoe6/Ac.

297. The segregation analysis of transgene phenotype indicates the presence of two functional loci in GS40/90pHoe6/Ac but does not account for non-functional transgenic sequences potentially inserted into additional genomic location (i.e. third transgenic locus). An inheritance study, based on the presence or structure of transgenic sequences in progeny plants should be conducted to confirm that only two transgenic loci are present in GS40/90pHoe6/Ac (see comment 3 in accompanying document).

298. EC062 – Attachment 3: The text cross-reference annexes that are not included in this attachment. This reviewer assumed that annex 8 and 9 corresponded to attachment 13 and 14.

299. EC062 – Attachment 13: Only a partial sequence of the pHoe6/Ac vector (including the T-DNA region and surrounding vector sequences) is provided. Attachment 13 (annex 8) does not provide information about the "insert" as stated in page 16 section 30a and 30b of attachment 3.

300. EC062 – Attachment 14: The Southern blot analyses do not provide definitive answers about the total number of copies of transgenic sequences introduced into GS40/90pHoe6/Ac. Further analyses (attachment 113, 114) will show that an additional copy to the two identified here are in fact inserted into the nucleus of GS40/90pHoe6/Ac.

301. EC062 – Attachment 74: This Expert disagrees with the comments in paragraph 6.1.3 "Transgenic construct in the GM plant". So far, the data presented only show that two functional loci are present in GS40/90pHoe6/Ac. No structural analysis of progeny has been conducted to demonstrate that only two transgenic loci were present and that each locus was structurally stable. The Southern blot analysis presented in attachment 14 (see above) does not provide information about the structure of each of the two transgenic loci. The use of the term "insert" is here again confusing as the sentence "It is shown that the insert is stable and follows standard Mendelian inheritance" means in fact that two functional loci have been identified and that the segregation of transgene phenotype (i.e. herbicide tolerance) across generations is compatible with two functional Mendelian loci. This is not completely informative about the structural stability or the number of transgenic loci (with the exception that there is at least two of them).

302. EC062 – Attachment 113: Page 70 of the PDF file and onwards, what is "transgen" In figures 1,2,3,5,6,7? Does "transgen" mean line GS40/90pHoe6/Ac?

303. EC062 – Attachment 113: This attachment contains, among many other documents, the study A59211 entitled "Southern analysis performed on the glufosinate tolerant 6Ac rape line". This Expert disagrees with the conclusion of this study that "no region outside the two border sequences of the binary vector pHoe6/Ac are integrated into the genome of the 6Ac rape line" stated page 69 of the PDF file. The Southern blot analyses undertaken in this study seem to have been conducted on a rape line containing only one copy of the CaMV35S:pat:35T expression unit integrated into the plant genome (the XbaI/XhoI digest hybridized with the pat gene produces only one band in figure 5). This is not compatible with the fact that GS40/90pHoe6/Ac contains at least two functional loci (i.e. at least two copies of the expression unit). Further molecular analyses should be conducted using restriction enzymes which cut only once into the T-DNA and using the same set of probes 1,2,3,4 and pat.

304. EC062 – Attachment 114: Page 5 should read "three copies of the T-DNA are inserted in oilseed rape event falcon GS40/90 at two independent functional loci. It was shown that the expression of the insert is stable and follows Mendelian inheritance". In the absence of molecular analysis of the progeny plants, the exact number and stability of transgenic loci cannot be established.

305. EC062 – Attachment 114: The full sequence of the transgenic locus EE1 is provided with flanking genomic sequences providing a full and optimal characterisation of this locus.

306. EC062 – Attachment 114: The sequence of the transgenic locus EE2 should be provided, especially as it contains a combination of two T-DNAs. This reviewer could not locate the southern blot analyses underlying the architecture proposed for EE2.

307. Conclusion – Response to Question 12: Since the first submission in 1996, the following molecular information about GS40/90pHoe6/Ac has been established:

- GS40/90pHoe6/Ac contains two independent functional loci, named EE1 and EE2;
- The locus EE1 and its plant genomic flanking regions have been fully sequenced and characterised. One copy of the CaMV35S:pat:35T expression unit is present in EE1;

- The locus EE2: A structural organisation involving two CaMV35S:pat:35T expression units in tail-to-tail orientation has been proposed and plant genomic flanking regions have been sequenced.

308. For a full molecular characterization to satisfy all regulatory requirements, this Expert would suggest that more information is still required about:

- The locus EE2: the Southern blot data supporting the structural organisation of this locus could not be found by this reviewer. It would be desirable to sequence the EE2 locus in order to provide the same type of complete information already available for the EE1 locus.
- The molecular analysis of GS40/90pHoe6/Ac for the presence of unwanted backbone sequences needs to be re-visited as inconsistencies appear in the current analysis (see attachment 113 – the plant analysed seems to contain only one pat transgene copy and not three as expected from GS40/90pHoe6/Ac).

309. The structural analysis of progeny plants is required to ensure that no additional transgenic sequences have been integrated in other genomic locations and that EE1 and EE2 are structurally stable across generations.

310. Additional information may have submitted to the EC by the company. Is so, it should be examined to see if it answers some of the requests formulated above.

**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?**

**Dr. Nutti**

311. According to the International Plant Convention's (IPPC) International Standard for Phytosanitary Measures (ISPM 11) in EC-130, the definition of pest is "Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products". In my opinion, Bayer oilseed rape (Falcon GS40/90) cannot be qualified as a potential "pest", according to this definition.

**Dr. Andow**

312. This question is a special case of the general case covered in my answer to question 6. My answer to question 6 holds in this special case. Specifically, it appears that on the issue of persistence that the SCP did not explicitly consider the issue of release rate or scale, nor did it determine whether contamination of conventional varieties was an injury to plants. Indeed, the data presented by the notifier, the argument of the notifier, and the argument implicit in the SCP Opinion all suggest that scale was not considered. Hence the SCP Opinion must be considered incomplete for allowing a determination of "pest" status. I conclude that the pest status of Falcon GS 40/90 cannot be determined based on the information before the Panel.

**Dr. Snow**

313. I conclude that in some situations, this product qualifies as a potential "pest", as I discuss in Answer 6. If the herbicide glufosinate becomes used more widely, as expected, volunteers and weedy

Brassica rapa that develop resistance could qualify as worse "pests". However, this problem should be manageable over the short term by using other methods of weed control.

**Dr. Squire**

314. In that oilseed rape as a volunteer or feral plant is a pest as defined in ISPM 11, e.g. a weed competing with a crop for resources, and having other potential influences, the Bayer oilseed rape (Falcon GS40/90) has the potential to be a pest, since it would leave volunteer and feral descendents. Whether it would be more or less a pest than other forms of oilseed rape depends on whether it would be sprayed as a volunteer or feral with the herbicide glufosinate ammonium (see Responses 6b-c). That would be context-specific, e.g. whether farmers in the region or country used this specific herbicide widely or repeatedly in fields.

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

**Question 14: Given the information before the Panel, including the conclusions of the EC SCP (EC-63/At.54) in relation to the potential persistence or invasiveness of Bayer hybrid oilseed rape (MS8/RF3), would this product qualify as a potential pest according to ISPM 11? If so, are the proposed post-market monitoring plans consistent with the monitoring and review principles described in ISPM 11?**

**Dr. Nutti**

315. According to the International Plant Convention's (IPPC) International Standard for Phytosanitary Measures (ISPM 11) at EC-130, the definition of pest is "Any species, strain or biotype of plant, animal or pathogenic agent injurious to plant or plant products". In my opinion, Bayer oilseed rape (MS8 / RF3) cannot be qualified as a potential "pest", according to this definition.

**Dr. Andow**

316. Regarding the definition of potential pest, this question is answered more generally in my response to question 6. That answer applies to this particular case. Specifically, it appears that on the issue of persistence that the SCP did not explicitly consider the issue of release rate or scale, nor did it determine whether contamination of conventional varieties was an injury to plants. Indeed, the data presented by the notifier, the argument of the notifier, and the argument implicit in the SCP Opinion all suggest that scale was not considered. Hence the SCP Opinion must be considered incomplete for allowing a determination of "pest" status. I conclude that the pest status of MS8/RF3 cannot be determined based on the information before the Panel.

317. The need for risk management and monitoring was outlined in the SCP (EC-63/At.54, 19 May 1998) as follows:

2. The Committee was also of the opinion that the potential transfer of the herbicide resistance gene to wild Brassica relatives is a new issue in Europe in view of the limited scale of release to date. The Committee has examined the available evidence from monitoring and research programmes to date. After evaluating all the information available to the Committee, it was concluded that herbicide-tolerant volunteers that may appear would be canola plants and not wild Brassica relatives. Such herbicide-tolerant volunteers could be controlled in subsequent crops by

conventional agricultural methods. The Committee recommends that the introduction of herbicide-tolerant crops should be accompanied by:

- i) an agreed code of practice for the particular modified crop involving the active participation of the notifier to promote best practice by farmers.
- 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.

318. The SCP is recommending a "code of practice" in order to ensure that herbicide-tolerant volunteers are eliminated. In other words, the purpose of the risk management measure is to eliminate volunteers as they occur. In addition, it is recommending research design and implement monitoring to detect herbicide-tolerant volunteers and herbicide-tolerant weeds in the EU.

319. In addition, the objectives of Annex VII, Directive 2001/18/EC also are relevant and are acknowledged as such by the notifier (EC-63/At.124, p. 6).

320. The notifier proposes a two-part monitoring system: case-specific monitoring for HT volunteers and HT weeds, and general surveillance for unanticipated effects (EC-63/At.124, p. 7ff). These parts are intended to meet the concerns expressed by the SCP and the objectives of Annex VII.

321. Section 3.6.1 of ISPM 11 describes the monitoring and review of phytosanitary measures. This concentrates on the principle of "modification", and is repeated here.

### **3.6.1 Monitoring and review of phytosanitary measures**

The principle of "modification" states: "As conditions change, and as new facts become available, phytosanitary measures shall be modified promptly, either by inclusion of prohibitions, restrictions or requirements necessary for their success, or by removal of those found to be unnecessary" (ISPM N° 1: *Principles of plant quarantine as related to international trade*).

Thus, the implementation of particular phytosanitary measures should not be considered to be permanent. After application, the success of the measures in achieving their aim should be determined by monitoring during use. This is often achieved by inspection of the commodity on arrival, noting any interceptions or any entries of the pest to the PRA area. The information supporting the pest risk analysis should be periodically reviewed to ensure that any new information that becomes available does not invalidate the decision taken.

322. This suggests that an important part of the risk management measures applied to GMHT crops should include a process of review of the necessity of the management measures. Additional general issues are discussed in my response to question 6.

323. The notifier has proposed a review process in the monitoring plan (EC-63/At.124, p. 21), which is repeated here.

### Review

This monitoring plan will be revised along with the expertise acquired. The monitoring plan and its associated methodology will be reviewed at the end of each growing season and updated as necessary in light of results obtained during the monitoring programme. Reviews will examine the effectiveness and efficiency of data measurements and collection in order to be able to improve the quality of the monitoring programme.

324. In addition, the notifier proposes to terminate case-specific monitoring after five years.

325. Thus, I conclude that the proposed post-market monitoring plans are consistent with the monitoring and review principles described in ISPM 11. The proposed plans are the most thorough and well-considered of any of the monitoring plans in the material before the Panel.

326. However, I also believe that the proposed plan could be much improved so that it is not merely consistent with ISPM 11 but actually meets the full intent of ISPM 11.

### Dr. Snow

327. I conclude that this product could qualify as a potential pest, as I discuss in Answers 6 and 13. If the herbicide glufosinate becomes widely used, as expected, glufosinate-resistant volunteers and weedy Brassica rapa could become worse pests. However, this problem can be managed using other methods of weed control. (note: the EC SCP conclusions about risks dealing with gene flow, volunteers, and wild relatives are the same as those for Bayer oilseed rape, above, with the same shortcomings; see Answer 11).

328. The ISPM 11 monitoring and review principles (section 3.6.1) discuss the need to modify phytosanitary measures as needed when new information becomes available. The post-marketing monitoring plans that are proposed by the notifier are consistent with these principles. If implemented, these plans would be useful for detecting the spread of glufosinate resistance to volunteer plants and wild relatives.

329. Caveat – The monitoring plans do not address broader concerns about monitoring for changes in biodiversity in and around farmland as a result of more efficient and extensive weed control, and they do not provide unique identifying markers for monitoring gene flow (e.g., these issues are discussed in the May 2004 Danish position; EC-063 At. 173).

### Dr. Squire

330. The basic answer is similar to that given for Q.13. A few statements need comment, however. The EC SCP (EC-63/At.54, section 6.3.1) gave the opinion that "rape is a poor competitor" and "not regarded as an environmentally-hazardous colonising species", and while the latter is still probably acceptable in semi-natural habitats, the continued widespread persistence of oilseed rape in and around arable fields, both from original seed and from re-seeding, points to its competitive ability being greater than "poor" but similar to many second-order weeds (i.e. weeds that are less important than the most aggressive few species but are still important). The proposed post-marketing monitoring plans (EC-63 At.060) are generally consistent with the principles in ISPM 11, but do not give specific detail on, say, how a field is to be sampled to estimate % GM presence among volunteers with a stated degree of certainty (see Responses 6e and 9).

**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?**

**Dr. Nutti**

331. Based on the information provided by the applicant (EC-63/At.01 to 176) and the analysis done by the EC SCP presented in CDA-35A and EC-063/At.54, the information presented in CDA-56 and following the Codex Guidelines, my understanding is that the request by the Italian CA (EC-63/At.87) was not necessary to ensure the conclusions for the safety assessment of the newly expressed protein in the food chain. As pointed out by the EC SCP in item 6.2 – Safety Aspects, there are no antibiotic resistance marker genes in this GMO. The bargene is controlled by a plant promoter which is not functional in bacteria. Consequently, its expression in the unlikely event of transformation would not occur. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms or human or animal cells, the probability of which is remote, no negative effects are expected because the only known substrate of the enzyme phosphinothricin acetyltransferase PAT is the herbicide glufosinate ammonium.

332. The PAT protein has been detected in very low amounts in dry seed (0.1 mcg/mg seed protein). As almost no protein is present in the oil extracted from the plants, the risk for human consumption is non-existent. The amounts of PAT (the new protein) present in the seed-meal fed to animals would be too low to cause even theoretical concern. The low amount of PAT protein in vegetative tissues of the plants is an additional guarantee of safety in case of occasional consumption of the green parts of the plant by farm or wild animals. The low levels of PAT protein and the weight of evidence available elsewhere concerning the safety of PAT leads the Committee to conclude that there is no significant risk to humans or livestock following the ingestion of the gene product.

333. Compositional analysis were carried out on the seed harvested from the original plant lines, their hybrids, and lines developed from them by multiple backcrosses to different oil seed rape varieties from field trials in Europe and North America, where locally adapted commercial varieties were used as controls. Information on fat content, glucosinolate levels, fatty acids profiles (including erucic acid), vitamin E and mineral content showed no differences which could be attributed to the genetic modification, leading to the assumption that no significant changes in nutritional value have been identified.

334. I agree with item 7 of the Overall Assessment, where the EC SCP states that there is no evidence to indicate that the placing on the market of hybrid seed of sweet rape (consisting of crossing of parentals derived from genetically modified sweet rape lines MS8 and RF3) with the purpose to be used as any other sweet rape is likely to cause any adverse effects on human health. Based on the scientific evidences presented, my understanding is that the new protein is safe for consumption in the food chain.

**Dr. Andow**

335. In EC-63/At.87, 14 March 2000, the Italian CA requested the following information.

Referring to the conclusion of the last meeting of the Regulatory Committee under Directive 90/220/EEC, Italy would be very pleased to receive from the notifier more information concerning the assessment of the effect of the genic product on the

biogeochemical cycles and on food chain and on the spreading of the gene due to the possibility of crossing between the PGM and wild species.

336. This is an identical request for information on the same date as for Falcon GS 40/90 (EC-62/At.95). Even without looking into the specifics of this case, my general comments on this request to the notifier of Falcon GS 40/90 in my response to question 10 in paragraph 252 also holds in this case.

337. The Italian CA made the request on 14 March 2000. The notifier provided an updated notification on 10 November 1999, which was circulated to the CAs on 13 December 1999. The notifier provided additional information on 25 February 2000 and 1 March 2000. Except for the dates that additional information was provided, this is the same timeline as Falcon GS 40/90, and my conclusion in paragraph 256 holds here.

**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?**

**Dr. Healy**

338. Following receipt in September 1996 of the notification from Plant Genetic Systems (PGS, now Bayer Crop Science) seeking approval for oilseed rape lines MS8 and RF3, the lead CA (Belgium) expressed a favourable opinion in January 1997. The dossier was then sent to the European Commission and distributed to other Member States for comment. Supplementary information from PGS required by Member States was added to the dossier, which was submitted to the SCP for assessment. In May 1998, the SCP also expressed a favourable opinion on MS8, RF3 and hybrid MS8xRF3 lines with respect to human health and safety.

339. Background: The two oilseed rape lines under consideration, MS8 and RF3, both contain a bacterial gene, *pat*, that confers tolerance to the herbicide phosphinothricin, also known as glufosinate-ammonium. The presence of the *pat* gene enables the rape plants (*Brassica napus*) to produce an enzyme, phosphinothricin acetyl transferase (PAT), which chemically inactivates the herbicide, allowing the plants expressing the PAT protein to function normally in the presence of the herbicide. The *pat* gene was used as a selectable marker gene in the individual lines and also to confer tolerance to commercial applications of glufosinate-ammonium herbicide in MS8/RF3 hybrids. There were no other marker genes used and there are no antibiotic resistance genes in either MS8 or RF3.

340. In conjunction with the herbicide tolerance trait, MS8 contains another bacterial gene, *barnase*. Expression of *barnase* in specific parts of the flower at a particular developmental stage gives rise to plants that do not produce pollen (male-sterile, MS). The *barnase* gene prevents pollen formation by producing a non-specific ribonuclease that destroys the tapetal cell layer in which it is expressed.

341. The RF3 line contains the corresponding bacterial gene, *barstar*, as well as the herbicide tolerance trait. Expression of the *barstar* gene in the same floral tissues, the tapetal cells, produces a protein that specifically binds and inactivates the *barnase*, thereby restoring normal pollen production. Hybrid MS8/RF3 plants are phenotypically normal and fully fertile. The primary objective of the modifications in MS8 and RF3 is to generate pollination-controlled parental oilseed rape lines with superior agronomic performance for use in a breeding system that generates hybrids with significantly higher seed yields.

342. Molecular characterisation data: PGS submitted a range of company studies on the molecular characterisation of MS8 and RF3 lines in its original dossier. These data were consistent with the approach generally considered appropriate at the time for the assessment of the safety of transgenic crop plants and derived food products intended for human consumption.

343. Descriptions of the plasmids pTHW107 and pTHW118 used to generate the MS8 and RF3 lines respectively were provided, including the size, source and function of the transgenes and their regulatory elements. In addition to standard Southern blot hybridisation analyses that generate information on the number and size of insertions, the data included DNA primer sequences, PCR methods for analysing the specific insertion events, and detailed DNA sequence analyses of the integration region in both lines. The CA (Belgium) declared in 1997 that the data provided showed that the transgenes in lines MS8 and RF3 are not inserted into a known functional plant gene (EC 063, attachment 007).

344. Approximately 4900 base pairs (bp) of the inserted DNA sequence in MS8 was provided, showing exact similarity with the transforming plasmid sequence between the left and right borders of the T-DNA, with no deletions or rearrangements. Flanking nucleotide sequence was provided at the 5' end (864 bp) and the 3' end (357 bp) of the inserted DNA. These flanking sequences were analysed for homology with known sequences. Part of the 5' flanking sequence showed some degree of similarity with published sequences from *Arabidopsis thaliana* chromosomes 5 and 3. However, no homology with known sequences was found using the 3' flanking sequence.

345. The DNA sequencing analyses of RF3 showed that the inserted DNA corresponds exactly with the transforming plasmid, however from the data it was evident that an inversely-oriented second partial fragment of T-DNA was present at the left border of the introduced DNA at the same insertion site. The second copy includes a functional part of the anther specific TA-29 promoter, the coding region of the barstar gene, 3' nos termination sequence and a non-functional part of the PssuAra with the T-DNA sequence ending 750 bp upstream of the ATG initiation codon of the bar gene. Detailed nucleotide sequencing across the integration sites generated 1077 bp at the right border, 1457 bp at the inverted repeat junction, and 1501 bp at the left border. Approximately 8900 bp corresponding to the entire RF3 insertion event was determined. Flanking nucleotide sequence was provided at the 5' end (812 bp) and at the 3' end (1275 bp) of the inserted DNA. These flanking sequences were analysed for homology with known sequences. Parts of the 3' flanking sequence showed some degree of similarity with published sequences from *Arabidopsis thaliana* chromosomes 1 and 5. However, no significant homology with known sequences was found using the 5' flanking sequence.

346. Conclusions of the Safety Assessment: The SCP considered in 1998 that the data submitted in the dossier was satisfactory concerning information on the transformation techniques, vector constructs, insertion event in the transgenic plants and transgene and cryptic gene expression. The overall assessment and conclusion of the SCP was that 'there is no evidence to indicate that the placing on the market of hybrid seed of swede rape (consisting of crossings of parentals derived from genetically modified swede rape lines MS8 and RF3) with the purpose to be used as any other swede rape is likely to cause adverse effects on human health and the environment'.

347. Additional Information Sought by Lead CA: Although junction regions at the sites of integration in the transformed lines were separately cloned and sequenced, there was minimal DNA sequence data generated from the wildtype isogenic lines at the corresponding locus. Consequently, the submitted homology searches were performed using sequence data obtained only from the transgenic lines. Subsequently, molecular details of the flanking regions were not considered to be adequate by the lead CA and the results of further PCR and sequence analyses were sought.

348. Impact of Additional Information on the Conclusions of the Safety Assessment: 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 rationale for this view are:

- Finely detailed molecular analysis of a defined chromosomal region in commercially adapted plant lines such as oilseed rape is likely to reveal minor nucleotide differences between various lines that have no significance with respect to the safety of derived food products. In the absence of an equivalent nucleotide analysis from a number of commercial food-producing oilseed rape lines, these data do not contribute substantially to the bank of information that is necessary to adequately characterise MS8 and RF3 at the molecular level. Moreover, it is not clear how such data should be interpreted in an assessment of transgenic plants.
- The data provided at that point in time (i) allowed adequate description of the insertion events in both transgenic lines, (ii) was sufficiently detailed, presented clearly and of acceptable quality, and (iii) was consistent with a generally accepted approach to the molecular characterisation of transgenic crops used for food production.
- 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.

349. Conclusion: The totality of information provided in the dossier to that point in time contained sufficient information to support the validity of the conclusions of the safety assessment without the need for the additional molecular characterisation data requested in the letter of the Biosafety Council dated 28 March 2002.

**Dr. Snape**

350. The comments below relate to Questions 16 and 18 concerning the molecular characterisation of exhibit EC-063. These were examined relative to the EFSA-Q-2003-005 guidance document produced by the Scientific Panel on Genetically Modified Organisms.

351. Oilseed rape event Ms8 was produced, using plasmid pTHW107, by means of Agrobacterium-mediated gene transfer. The T-DNA of the plasmid pTHW107 used for transformation carries two expression units: one contains the bar gene, conferring resistance to the herbicidal compound glufosinate and one contains the barnase gene to engineer male sterility. A barstar gene (driven by a bacterial promoter) is also present in the backbone of pTHW107. Oilseed rape event Ms8 is described as containing one functional transgenic locus containing one copy of the pTHW107 T-DNA.

352. Oilseed rape event Rf3 was produced, using plasmid pTHW118, by means of Agrobacterium-mediated gene transfer. The T-DNA of the plasmid pTHW118 used for transformation carries two expression units: one contains the bar gene conferring resistance to the herbicidal compound glufosinate and one contains the barstar gene to engineer fertility restoration. An additional copy of the barstar gene (driven by a bacterial promoter) is also present in the backbone of pTHW118. Oilseed

rape event Rf3 is described as containing one functional transgenic locus containing two copies of the pTHW118 T-DNA in inverted orientations, one copy of the T-DNA being intact the other one being partially deleted.

353. Information relative to the molecular characterisation of Ms8 and Rf3 has been found primarily in EC-063 attachments 4, 19, 20, 43, 54, 98, 114, and 115. A series of comments and questions are numbered below. This is followed by a conclusion proposing answers to Questions 16 and 18:

354. EC063 – attachment 001: (1) Contains only the table of content of the initial notification. The data corresponding to the molecular analysis in part1 section III and IV (pages 7 to 20) were not found by this Expert in subsequent attachments. When requested, this information was not available from WTO either. It is likely that some relevant information was initially submitted by the company. However in its absence this Expert cannot determine:

(1a) The sequence of pTHW107 and pTHW118

(1b) If oilseed rape event Ms8 contains only one copy of the bar gene and one copy of the barnase gene.

(1c) If oilseed rape event Rf3 contains only one copy of the bar gene and two copies of the barstar gene.

355. EC063 – attachment 019 and 020: Presents a PCR analysis suggesting the absence of Sm/Sp antibiotic resistance gene in Ms8 and Rf3.

356. EC063 – attachment 043: Presents a Southern blot analysis demonstrating the absence of vector backbone sequences into Ms8 and Rf3 including the barstar gene in Ms8.

357. EC063 – attachment 098: The transgenic locus in Ms8 and its plant genomic flanking regions have been fully sequenced and analysed providing an optimal characterisation of this locus. This transgenic locus contains one copy of the pTHW107 T-DNA.

358. The transgenic locus in Rf3 and its plant genomic flanking regions have been fully sequenced and analysed providing an optimal characterisation of this locus. This transgenic locus contains two copies of pTHW118 T-DNA in inverted orientations, one copy of the T-DNA being intact the other one being partially deleted.

359. Conclusion – Response to Questions 16 and 18: Additional information may have been submitted to the EC by the company (see point 1a, 1b, 1c above). Experts should examine if this information answers some of the requests formulated below.

360. Ms8: Since the first submission in 1996, the following molecular information about Ms8 has been established:

- Ms8 contains one transgenic locus (if confirmed by Southern analysis see point 1b).
- This locus and its plant genomic flanking regions have been fully sequenced and characterised. One copy of the pTHW107 T-DNA is present at this locus.
- Transgenic sequences outside the T-DNA of pTHW107 have not been integrated into Ms8.

361. This Expert concludes that no more information is required (if Southern analysis confirm point 1b). As Ms8 contains a single copy of the bar and barnase gene, the monitoring of transgene expression across generations provides a satisfactory estimation of the transgenic locus structural stability and inheritance.

362. Rf3: Since the first submission in 1996, the following molecular information about Rf3 has been established:

- Rf3 contains one transgenic locus (If confirmed by Southern analysis see point 1c).
- This locus and its plant genomic flanking regions have been fully sequenced and characterised. Two copies of the pTHW118 T-DNA in inverted orientations are present at this locus, one copy of the T-DNA being intact the other one being partially deleted.
- Transgenic sequences outside the T-DNA of pTHW118 to have not been integrated into Ms8.

363. In the opinion of this Expert, to satisfy all regulatory requirements, more information is still required about the structural analysis of progeny plants to ensure that the transgenic locus is structurally stable across generations. As Ms8 contains two copies of the barstar gene the monitoring of transgene expression across generation is not informative on the stability and inheritance of the transgenic locus.

**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?**

**Dr. Nutti**

364. A qualitative method available at the time. EC-063/At.141 asks for a quantitative event specific detection tool for elite events and hybrids. It is recognized that a qualitative detection method and reference materials were provided by the company. Based on the information provided at CDA-56, I can infer that there was no general validation of the method used by the notifier. So far, only DNA extracted from unprocessed raw material has been tested. It was observed that this detection method should also be demonstrated to work using other matrices. Further, the sensitivity of the detection method should still be assayed by testing the presence of the elite or hybrid events in mix samples. Finally, based on the given method, one cannot make a distinction between the hybrid and a mixture of the two parental lines. My conclusion is that the method presented was technically sufficient for the safety assessment, although it would have to be validated.

365. Although a quantitative method will provide relevant information for labelling and to the consumer, it is not necessary to ensure the validity of the safety assessment. It is important to point out that the EC SCP has considered that there is no evidence to indicate that the placing on the market of hybrid seed of oilseed rape (consisting of crossing of parentals derived from genetically modified oilseed rape lines MS8 and RF3) with the purpose to be used as any other oilseed rape is likely to cause adverse effects on human health.

**Dr. Healy**

Question 17 – part 1:

366. The transgenic oilseed rape lines MS8 and RF3 are developed specifically to generate high yielding seed produced by the MS8xRF3 hybrid. Food products intended for human consumption are therefore derived from the hybrid seed rather than from the individual parental lines. Novel transgenic proteins expressed in the hybrid plants include the PAT protein (herbicide tolerance), and the two bacterial proteins barnase and barstar (for pollination control in the parental lines). The patterns and levels of gene expression conformed to those predicted and intended by the modification process.

367. The SCP acknowledged that the dossier contained appropriate information on PCR detection methods for identification of MS8 and RF3 transgenic lines (Opinion, 19 May 1998).

368. Barnase and barstar: The barnase and barstar genes, derived from the bacterium *Bacillus amyloliquefaciens*, each encode a different small single-chain protein. Both of these proteins have been studied extensively as models for protein folding because of their small size, and there is an abundance of published scientific information relating to research work conducted since the early 1960s.

369. The barnase gene encodes a ribonuclease that is naturally secreted by the bacterium. Ribonucleases are ubiquitous in nature, and serve many biological functions. In this case, the secreted ribonuclease serves to protect the environment of the bacteria. Conversely, the barstar gene encodes a specific protein inhibitor of this ribonuclease. In the *Bacillus* species from which the two proteins are derived, the function of the barstar protein is to protect the organism from the otherwise toxic effects of its own barnase activity. This naturally occurring system is well studied and the interaction of the two proteins is known to be highly specific.

370. Under the control of a highly tissue-specific plant promoter from tobacco (PTA29), expression of barnase and barstar in the MS8xRF3 hybrids is restricted exclusively to the pollen producing cells in developing anthers and only when the plants are flowering. The two proteins are not expressed elsewhere in the plant, including the leaves, stems or seeds.

371. Gene expression data: For the parental RF3 line, barstar messenger RNA (mRNA) was barely detected in flower buds only, but not in any other plant tissues, including the seeds. As expected, because expression of the ribonuclease results in cell death, barnase mRNA could not be detected in any tissues from the MS8 line.

372. Expression of PAT protein: The enzyme responsible for herbicide tolerance, PAT, is mainly expressed in the green tissues of the plant. There is some expression in other tissues including the seeds, but at such low levels that specific enzyme activity was not detectable. Messenger RNA (mRNA) corresponding to the bar gene could be detected at extremely low levels in the leaves and flower buds of parental lines MS8 and RF3, but not in the seeds of the plants.

373. Hybrid MS8xRF3 would be expected to express higher levels of PAT than either parental line. Data were provided indicating that the levels of PAT expressed in the seeds of the individual parental lines were detectable using the specific Enzyme Linked Immunosorbent Assay (ELISA) system, with a detection limit of approximately 5 ng/ml. As expected, the level of PAT protein in various oil fractions of seeds derived from the MS8xRF3 hybrid is below the limit of detection.

374. Protein detection methodology: As well as detailed DNA-based detection protocols, the company provided protein-based detection and identification methodologies (ELISA and test kit) which were evaluated at the September 2001 meeting of the Scientific Committee "Transgenic Plants" of the Biosafety Council (EC 63-30). In its discussions of the information, the Committee raised several matters concerning the protocol for detection of the PAT protein using ELISA. Concerning amounts of PAT protein detected, there were questions raised on the use of the standard curve and on the limit of detection. It was also remarked that the detection methods proposed by the company were qualitative rather than quantitative.

375. However, quantitative immunoassay methods to detect novel proteins in foods derived from transgenic crops were under development during the late 1990's. The two most common tests are ELISA and lateral flow strips. Commercial immunoassay methods were reportedly available for detection and quantification of a number of transgenic proteins including the PAT protein in 2000<sup>59</sup>. As there are numerous transgenic food crops that have been genetically modified for tolerance to the glufosinate-ammonium herbicide, detection of PAT in the MS8xRF3 hybrids is not an event-specific detection method. Information supplied by Strategic Diagnostics Inc (Delaware, USA) indicates that their Lateral Flow test for oilseed rape was commercially available in April 2002.

376. Conclusion: The available evidence indicates that immunoassay-based detection methods suitable for the detection of PAT protein in MS8xRF3 hybrid transgenic plants were commercially available as early as 2000.

Question 17 – part 2:

377. Additional information regarding the provision of an event-specific quantitative detection tool was sought on 25 February 2003 (EC 63-141). The request for a detection method that is "event-specific" directly implies the use of a DNA-based method rather than a protein-based method, which can be quantitative but in this case is not event-specific.

378. The company provided both DNA-based (PCR primers and protocols) and protein-based methods (ELISA and test kit) for detection and identification of its transgenic lines. This information together with reference material was used in an independent laboratory to evaluate the detection protocol. The results of this evaluation indicated that the DNA-based PCR detection methods were reproducible and valid for the transgenic oilseed rape lines MS8 and RF3.

379. The provision of event-specific oligonucleotide primers for unambiguous identification of transgenic plant lines readily enables use of qualitative PCR methods, but also allows development of quantitative PCR technology according to plant species. 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. Moreover, it is generally recognised that quantitative protein-based methods of detection are more directly accessible.

380. Quantitative detection is an integral part of enforcement capabilities especially concerning labelling regulations. However, it is not a necessary component of a safety assessment. Given the comprehensive nature of the information and material provided in the updated dossier regarding identification primers, protocols and plant-derived DNA reference material, and the existence of

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<sup>59</sup> International Life Sciences Institute (ILSI) Europe Novel Food Task Force in collaboration with the European Commission's Joint Research Centre (JRC) and ILSI International Food Biotechnology Committee, Joint Workshop: Method Development in Relation to Regulatory Requirements for the Detection of GMOs in the Food Chain, December 2000. Journal of AOAC International, Vol. 85, No. 3, 2002.

established quantitative protein-based detection methods, I consider that further information relating to quantitative event-specific detection tools for transgenic oilseed rape lines MS8 and RF3 is not necessary to ensure that conclusions of the safety assessment were valid.

381. Conclusion: Additional information regarding an event specific, quantitative detection method requested in February 2003 is not necessary to ensure that the conclusions of the safety assessment are valid.

**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?**

**Dr. Healy**

382. The original dossier was supplemented with additional molecular characterisation data and other information to conform with particular requirements of the Belgian Biosafety Council, the SCP and Member States. The totality of the data and information provided allowed a comprehensive assessment of the safety of food and feed derived from transgenic oilseed rape lines MS8, RF3 and hybrids, as well as the potential environmental impact. Previously, both the Biosafety Council and the SCP had declared that these lines do not pose human health and safety concerns, and the additional information sought between 1998 and 2002, and subsequently provided by the company, had not altered these favourable conclusions.

383. Additional Information Sought by CA: The new Belgian guidelines on molecular characterisation were developed over a period of time culminating in the final draft version (version 6) being distributed for comments to the Scientific Committee in November 2001. This version was submitted to the members of the Biosafety Council for final approval at the meeting of February 2002 (EC-63/At.105). Additional information was sought 'in order to bring the molecular data in the file into line with the new Belgian guidelines on molecular data: evaluation of the presence and functionality of new chimeric ORFs via bioinformatics on the sequence of the insert and the flanking areas'.

384. It is noted that more detailed requirements concerning molecular characterisation data were required by Belgium prior to September 2001. In response, an extended package of information was provided by the company, and this additional information was evaluated at two subsequent meetings of the Scientific Committee "Transgenic Plants" on 27 September 2001 and 7 February 2002. After some modifications, the Scientific Committee approved the information supplied in these additional documents. It is also noted however that the new Belgian guidelines were developed in parallel with the ongoing assessment of the dossier and requests to the company for further molecular information. It is surprising therefore that the information necessary to comply with the new molecular characterisation guidelines was not requested until July 2003.

385. Impact of Additional Information on the Safety Assessment: 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. In the specific case of the frequently updated dossier on MS8, RF3 and hybrids, it is clear from the wording of the request to the company that the additional information sought is for the purpose of "simply" making the file consistent with the current Belgian guidelines on molecular data. This additional information does not add substantially to the weight of

evidence that supports the lack of human health and safety concerns associated with the use of the transgenic oilseed rape.

386. In this constantly changing environment, the data requirements for the molecular characterisation of transgenic crops are not static. In general, there is a recognised need to constantly monitor the progress of technical advances and adapt regulatory guidelines accordingly as techniques move from the highly experimental area to more validated and established methodology. It is acknowledged for example that newly evolving research fields such as proteomics and metabolomics may play a strategic role in the future assessment of food products derived from transgenic organisms.

387. Conclusion: The additional information sought by the CA in the email of 14 July 2003 (EC 63-144) on molecular data was requested to update the dossier to new guidelines and was not necessary to ensure the conclusions of the safety assessment were valid.

**Dr. Snape**

388. See answer given to Question 16

**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?**

**Dr. Andow**

389. The lead CA requested the following information (EC-63/At.149, 10 October 2003): "The Council found a shortcoming highlighted in the report to be sufficiently serious in terms of Annex 2, D2 points 4, 5, 7, 8, 9 of Directive 2001/18: the data on file are not precise enough to inform the Council properly about the impact of commercial crops of Ms8 or Rf3 or their hybrids, in the presence of glufosinate on the parameters set in the aforementioned points in agricultural ecosystems. Further data are awaited concerning: farmland diversity (macro-fauna, weed flora and microbial soil ecosystem), food web integrity (trophic structure), population dynamics of key species, life-cycles, etc."

390. The lead CA is referring to Directive 2001/18/EC Annex 2, section D2, which is reproduced here:

D2. In the case of genetically modified higher plants (GMHP);

4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable);

5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.;

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed;

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s);

9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

391. The notifier's original response to these Annex 2 guidelines is given in EC-63/At.112, 14 January 2003.

392. The notifier's full response to point 4<sup>60</sup> was "This is not applicable to glufosinate tolerant oilseed rape." This is an insufficient response. At a minimum there should be an argument as to why the point is not applicable. I conclude that the request by the lead CA for more information on point 4 was necessary to ensure that conclusions of the safety assessment were valid.

393. The notifier's response to point 5 covers 4 pages.<sup>61</sup> The notifier concludes

- Scientific experts confirm that the horizontal gene transfer from the GMHP to bacteria is extremely unlikely although theoretically it cannot be totally excluded. In the very unlikely case where such a transfer would occur this would have no impact since the transgenes involved would not provide a selective advantage.
- Scientific experts also confirm that the GM OSR has no effect on honeybees and epigeal predatory arthropods of oilseed rape.
- There are no identified potential effect of the GMHP on birds and small mammals.

394. I believe that the notifier's first conclusion is well supported by the data and arguments they muster. I believe that the data on epigeal predators is weak, but even with additional data, I am not convinced that a major adverse effect will be identified. The data on birds and rabbits are also weak, but again, I am not convinced that additional data will reveal a major adverse effect.

395. As there are literally hundreds of species of non-target organisms that could be tested, it can always be argued that more species should be evaluated. Indeed, several important ecological functional groups were not evaluated, including parasitoids and detritivores. Without a specific reason, it is difficult to argue that testing a parasitoid is necessary for the risk assessment. A case could be made that a detritivore would have been a better choice for study than epigeal predators. However, if this was the specific concern of the lead CA, it would have been appropriate to clarify the point. Hence, I conclude that the request by the lead CA for more information on point 5 was not necessary to ensure that conclusions of the safety assessment were valid.

396. The notifier's response to point 7 covers 3 pages.<sup>62</sup> The notifier argues that there is no reason to believe that the feed value of GMHT oilseed rape will be different from conventional oilseed rape, provides a summary of data on the major nutrients of seed and meal, and report the results on a rabbit and chicken feeding study. It is possible that the compositional studies are not adequate or the wrong

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<sup>60</sup> EC-63/At.112, p. 28, 14 January 2003.

<sup>61</sup> EC-63/At.112, pp. 29-32, 14 January 2003.

<sup>62</sup> EC-63/At.112, pp. 37-39, 14 January 2003.

species have been used for feeding studies. I am unable to conclude that the request by the lead CA for more information on point 7 was necessary or not necessary to ensure that conclusions of the safety assessment were valid.

397. The notifier's response to point 8 is one page.<sup>63</sup> The notifier claims that oilseed rape does participate in biogeochemical cycles, therefore there are no expected effects on biogeochemical cycling. The notifier further supports this view by reporting that there have been no observed effects on rhizosphere bacteria and no observed effects on the plants growing in the field the year following a crop of GMHT oilseed rape.

398. The notifier is mistaken to claim that oilseed rape does not participate in any biogeochemical cycles. The root and leaf litter participate in carbon cycles, and it may have subtle effects on phosphorous cycles, as it will reduce proliferation of mycorrhizal fungi relative to most other plants. It is likely that participation in carbon cycling is one of the main ways it participates in biogeochemical cycles. A simple litter degradation study would determine if it is likely to have any effects on carbon cycling. Hence, I conclude that the request by the lead CA for more information on point 8 was necessary to ensure that conclusions of the safety assessment were valid.

399. The notifier's response to point 9 covers two pages.<sup>64</sup> The notifier argues

The only change in cultivation techniques used on the herbicide tolerant (hybrid) oilseed rape is the possibility to use glufosinate ammonium as selective herbicide. However, glufosinate-containing herbicide can be used on the crop only if they are registered for this specific use in the different Member States according to Directive 91/414/EEC. The safety assessment and the assessment of the impact of the herbicide use on the environment are done in the said 91/414 application.

400. The notifier argues that except for herbicide use, other cultivation practices are unlikely to change. This contrasts sharply with claims by others that GMHT crops are likely to result in changes in tillage practice. The notifier does not address this possibility in detail and states only that soil preparation practices will be identical. This seems to be an insufficient argument. In addition, 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.

401. The lead CA requested information on 10 October 2003. The notifier submitted the material to be reviewed on 14 January 2003. The 269 days that elapsed seem excessively long to complete the review and articulate the questions.

**Dr. Snow**

402. The vagueness of this request and the lack of related information from the notifier make it difficult to provide a clear answer to this question from the Panel.

403. The lead CA asked for information about effects on "farmland diversity (macro-fauna, weed flora and microbial soil ecosystem), food web integrity (trophic structure), population dynamics of key species, life-cycles, etc." (EC-63/At. 149 translation). Although this request seems to ask for

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<sup>63</sup> EC-63/At.112, p. 40, 14 January 2003.

<sup>64</sup> EC-63/At.112, pp. 41-42, 14 January 2003.

some unnecessary data (and the request is quite vague), several Member States were concerned about the ecological effects of more intensive herbicide use. I assume that this is the focus of concern – that more information was requested to determine the effects of cultivating new herbicide-tolerant crops on the weed plants that support insect and animal populations in farmland habitats in these countries.

404. Realistically, this is a difficult set of questions to answer, regardless of whether one uses basic theory or empirical research. As noted above, the indirect effects of introducing herbicide-tolerant crops to countries like the UK are still being investigated, so in this sense the requested information could be considered as necessary. However, it is not feasible to obtain scientific data on all of the processes listed by the CA without extensive multi-year studies. Even the Farm Scale Evaluations are too short and too small-scale to answer these questions (e.g., Andow 2003, Squire et al. 2003). Adverse effects on flora and fauna are mentioned in SPS and ISPM 11, but it is not possible to test for all of these effects prior to deregulation. Instead, scientific committees typically use their best judgement to recommend approving or denying applications for marketing.

#### **Dr. Squire**

405. The updated environmental risk assessment is comprehensive on topics for which data are available. The further information on ecological effects requested by the lead CA was clearly not available, and could only be in existence if there had been either large scale experimental measurements on the habitats typical of where the crop would be grown commercially or specially commissioned experiments on trial crops, as in the UK's Farm Scale Evaluations. Since cropping with this variety and its herbicide has potentially new effects on the arable flora (i.e. different from those of other oilseed rape varieties with other herbicides), which may be severely depleted in any case, it is legitimate to ask what such effects might be. However, the general matter of the need for a comparator is relevant here (Notes, paragraphs 32-35). Surely it is incumbent on both sides in the argument to proffer their standard or comparator against which the new technology should be judged.

**Question 20: Are the conclusions and recommendations in the Report of the Group of Experts Mandated by the Belgian Biosafety Advisory Council (EC-63/At.167 pp.20-21) regarding the agricultural guidelines proposed by the applicant consistent with the Belgian Biosafety Advisory Council's conclusion that the agricultural guidelines are "impracticable, hardly workable and hard to control"?**

#### **Dr. Andow**

406. The Belgian Biosafety Council concluded (EC-63/At.166, 27 January 2004) as follows

Concerning the environmental risk, the Biosafety Advisory Council is of the opinion that some risks associated with these GM crops will be reduced provided that the following conditions are strictly applied:

- The agricultural guidelines must be (1) adapted to the recommendations of the experts and to the recommendations made by the Member States due to differences in agricultural practices and landscapes, (2) applied by all the operators supported by appropriate legislation and control, and (3) continuously updated according to the new available scientific information.
- The post-market monitoring plan must (1) assess compliance with the agricultural guidelines, (2) assess and determine the effectiveness of the proposed risk management strategies of the agricultural guidelines in limiting

gene flow and its potential consequences and in preserving farmland biodiversity, (3) be adapted to the recommendations made by the Member States of the European Union due to differences in agricultural practices and landscape, (4) be continuously updated according to the new available scientific information and (5) the notifier must provide a more detailed description of the tasks and the kind of data that will be collected by different networks involved in the general surveillance. In this context the detection/identification method must be validated.

Furthermore, the Biosafety Advisory Council considers that presently, a number of recommendations of the agricultural guidelines are impracticable, hardly workable and hard to control in current agricultural practices, hence vertical gene flow and negative consequences on biodiversity may not be controlled.

407. The expert recommendations referred to are (EC-63/At.167, 3 February 2004, pp. 20-21)

408. **4.2.3. Agricultural guidelines**

Taking the importance of the agricultural guidelines into account, the Group of Experts recommends that the agricultural guidelines should be translated in the national or local language(s) of each MS of the EU. The guidelines should be readable and understandable by all the operators (e.g. farmers, seed suppliers) that will handle the seed, crop and product of MS8, RF3 and MS8xRF3. A widely comprehensible language should be used. Moreover, the agricultural guidelines should be adapted to the recommendations made by the Member States of the European Union because of differences in agricultural practices and landscape and be continuously updated according to the new available scientific information.

Given that the occurrence of spontaneous inter-specific crossings among oilseed rape, *Brassica rapa* and *Brassica juncea* crops has already been reported, the Group of Experts recommends that each farmer growing *B. napus*, *B. juncea* or *B. rapa* crops in the neighbourhood of the transgenic oilseed rape fields is aware of the agricultural guidelines.

Considering that seeds will be imported and transported and that seed spillage might occur, feral oilseed rape plants might establish and persist outside the fields (e.g. on roadsides, railways, wastelands, points of import). For this reason, the Group of Experts recommends that every operator should be informed on the appropriate management measures to be taken in the case of accidental seed spillage and the establishment of feral oilseed rape populations. It should also be made clear to whom these events must be reported. Where seed spillage occurs, the agricultural guidelines should recommend that the seeds must be swept or shovelled into sealed containers. In addition, seed spills should be recorded and monitored in subsequent years in order to allow appropriate control.

To limit seed spillage during transport and the potential establishment of feral herbicide-tolerant oilseed rape population, the agricultural guidelines should recommend transporting the seeds in closed and sealed containers.

To limit the development of multiple herbicide-tolerant plants through vertical gene transfer of the herbicide-tolerant trait, the agricultural guidelines should recommend that:

- the cultivation of transgenic oilseed rape with tolerance to other herbicides in the same or nearby rotation must be avoided,
- oilseed rape must not be grown on the same land more than one year in four.
- Given that reliance on a single mode of herbicide control exerts a high selective pressure on the weed population and volunteers, which may lead to the development of resistance to the concerned herbicide and that rotating active ingredients reduces resistance development, the agricultural guidelines should recommend that:
  - the glufosinate ammonium must be used in combination with other herbicides during the same rotation cycle,
  - the use of different crops tolerant to glufosinate ammonium must be avoided in the same rotation cycle,
  - integrated weed management systems should be applied.

Given that agricultural weeds become less abundant in fields where glufosinate ammonium is applied and that the environmental disbenefits of very clean fields might be judged unacceptable, uncropped fields and/or field margins and untreated field margin strips might be the only physical habitats to preserve these plants and the whole chain of living organisms living on and from these plants. Within those fields, margins or strips, the agricultural weeds and the accompanying fauna may find a place to survive, provided that the farmers allow them to. For this reason the agricultural guidelines should recommend farmers to preserve field margins (hedges, hedgerows, grassy borders, small woodlots and others), leave field margins unsprayed and limit herbicide drift. The agricultural guidelines should also recommend farmers to use band spraying if the negative impact of cleanliness is to be reduced.

To limit any selective pressure of the glufosinate ammonium on plants outside the field (e.g. herbicide-tolerant feral oilseed rape plants, wild relatives in field margins), the agricultural guidelines should recommend farmers to avoid herbicide drift in field margins according to the current principles of good agricultural practices.

409. The original proposed Agricultural Guidelines are in EC-63/At.123, 14 January 2003. I consider the question from the Panel to be ambiguous. The conclusions and recommendations in the Report of the Group of Experts (RGE) are about improving the original Agricultural Guidelines (OAG) proposed by the notifier. The conclusion by the Belgian Biosafety Advisory Council (BBAC) is also about the original Agricultural Guidelines. The stated question may be interpreted to be: do the conclusions and recommendations in the RGE support the conclusion by the BBAC that the OAG are "impracticable, hardly workable and hard to control"? Alternatively, the stated question may be interpreted to be: is there anything in the conclusions and recommendations in the RGE that contradict the conclusion by the BBAC that the OAG are "impracticable, hardly workable and hard to control"? I will assume that the stated question is not anything similar to: Are the OAG "impracticable, hardly

workable and hard to control," or are any of the conclusions or recommendations in the RGE "impracticable, hardly workable and hard to control"? I will address the first possible alternative here.

410. First, it should be clear that the RGE does not contain any conclusion similar to that of the BBAC, i.e., that the OAG are "impracticable, hardly workable and hard to control."

411. The RGE states

To allow safe and sound integration of MS8, RF3 and MS8xRF3 oilseed rape in Europe the notifier proposed agricultural guidelines giving guidance to farmers and seed suppliers. Since the experts considered that the guidelines would be crucial in limiting vertical gene flow and its potential consequences, it was strongly recommended that the practical mitigation measures as described in the guidelines would be followed.

412. This implies that some of the mitigation measures in the OAG are considered impracticable, as there would be no need to specify "practical mitigation measures" in the statement if all were considered practical.

413. The RGE also states

Moreover, it was proposed that the effectiveness of the proposed risk management strategies in limiting vertical gene flow (and its potential consequences) and in preserving farmland biodiversity should be assessed in the post-market monitoring plan. In addition, an independent farmers' audit to monitor compliance with the agricultural guidelines should be foreseen on a regular basis.

414. This is a critically important control point that was lacking in the OAG. This implies that the RGE considers some of the risk management measures proposed in the OAG to be hard to control.

415. The RGE organizes the recommendations for the Agricultural Guidelines along routes of escape and potential adverse effects. The OAG is organized around farm operations. The OAG emphasizes workability for farmers and seed distributors, while the RGE emphasizes workability for attaining the risk management objectives. Obviously, both must be met ultimately, but it is not clear from the BBAC conclusion what kind of workability is considered.

416. Thus the RGE supports the BBAC conclusion that the OAG are impracticable and hard to control. It is not clear if the RGE supports the BBAC conclusion that the OAG are hardly workable.

417. In passing, and not really on the point, I agree with the BBAC that parts of the OAG are "impracticable, hardly workable and hard to control," but would prefer to consider these parts as needing considerable improvement before they are acceptable. I also consider some of the recommendations in the RGE to be "impracticable, hardly workable and hard to control," and overall the Agricultural Guidelines will need additional work so that they accomplish the risk management goals.

#### **Dr. Squire**

418. The question is whether the words "impracticable, hardly workable and hard to control" used by the Belgian Biosafety Advisory Council are justified on the basis of the report by its Group of Experts. The Group of Experts does not use this wording as such but makes cautionary remarks that

the guidelines are not sufficient as they stand and should be extended and that there could be practical problems in successfully implementing them. Examples include –

- "each farmer growing *B. napus*, *B. rapa* and *B. juncea*, in the neighbourhood of the transgenic oilseed rape field is aware of the agricultural guidelines": this requires that non-GM farmers need to make themselves aware of GM issues, and that all farmers will be cooperative neighbours.
- "every operator should be informed on the appropriate management measures to be taken in the case of accidental seed spillage ...etc.": this in reality is probably unworkable, since many spillage events will go unnoticed, and there are such a large number of feral populations arising from such events.
- "seed should be swept or shovelled into sealed containers" and "(spillage sites) should be recorded and monitored in subsequent years": these are likely to be impracticable in commercial agricultural areas; the measures would place an intolerable burden on whoever was considered responsible if that could be decided (the grower, the transported, the buyer, the roads authority?).

419. Several other of the Group of Experts' recommendations are workable, but the above three examples would appear to justify the Belgian Safety Advisory Council's choice of words.

**Trifolium/Monsanto/Danisco Roundup Ready fodder beet (A5/15)**  
**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?**

**Dr. Nutti**

420. Based on the information provided by the applicant in EC-067/At.1 and the analysis done by the EC SCP (CDA-35A) and following the Codex Guidelines, my understanding is that the additional risk assessment provided by the notifier was not necessary in the first place. It does not address outstanding scientific concern since they are not related to the potential risks associated with this product.

421. It is important to point out that both the EC SCP and the Danish CA overall assessments have stated that there is no evidence indicating that the use of the 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.

**Dr. Andow**

422. The issue addressed in EC-64/At.104-105, 19 October 1999, was the existence and sequence of backbone DNA. This issue had not been addressed previous to this date for this transgenic fodder beet. The notifier indicated that they would provide information documenting that the backbone DNA was absent, but in the meantime would provide a risk assessment assuming the entire backbone DNA was present (EC-64/105, 19 October 1999). This risk assessment was not available in any of the materials before the Panel.

423. The issue addresses the possible activity of backbone DNA in the plant. If it exists and it becomes active, it could result in unanticipated phenotypes that could lead to adverse effects on human health or the environment.

424. Thus I conclude that the additional risk assessment would have addressed an outstanding scientific concern related to the potential risks associated with this product.

425. The request by the Dutch CA was on 4 August 1999, following the launching of the Inter-Service Consultation on 4 September 1998. The notifier provided additional information on 24 September 1998, 25 September 1998, 5 October 1998, 13 November 1998 and 5 March 1999. Counting from the date of the launching of the Inter-Service Consultation, the Dutch CA took 334 days. Counting from the date the notifier last provided additional information, the Dutch CA took 152 days. In either case, the number of days seems like a long time to make the response.

**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?**

**Dr. Nutti**

426. Based on the information provided by the applicant in EC-067/At.1 and the analysis done by the EC SCP (CDA-35A), I understand that there was no need, as far as food safety is concerned, for the request made by the Italian CA (EC-64/At.116) in order to ensure that conclusions of the safety assessment were valid.

**Dr. Andow**

427. The information requested by the Italian CA (EC-64/At.116, 14 March 2000) is as follows.

Referring to the conclusion of the last meeting of the Regulatory Committee under Directive 90/220/EEC, Italy would be very pleased to receive from the notifier more information concerning the transfer and the recombination of genes in natural conditions, on the efficacy of the use of the Roundup herbicide and on the evaluation of the possible crossing with spontaneous species.

428. Issues related to the transfer and recombination of genes in natural conditions and to the evaluation of possible crossing with spontaneous (wild?) species were addressed in the SCP Opinion (EC-64/At.83, 23 June 1998) and were addressed in the original notification.

429. It is possible that the Italian CA believed these assessments to be inadequate. If so, the CA should have indicated this in the question. However, as discussed below, this is unlikely.

430. Issues related to the efficacy of the use of glyphosate were not addressed in the SCP Opinion, but I fail to understand how such issues relate to a risk assessment, and the Italian CA provides no guidance. This question was NOT necessary to ensure that conclusions of the safety assessment were valid.

431. In the response to the Italian CA (EC-64/At.119, 12 July 2000), the notifier states that there are no new data in the document, only clarifications. Given that the Italian CA did not follow up on this response, I must assume that the response satisfied the Italian CA's concerns.

432. Thus, the issues raised by the Italian CA had been addressed in previous documents. Based on the detailed responses from the notifier in EC-64/At.119a, the question on possible crossing with spontaneous species was NOT necessary, but the question on transfer and recombination in natural conditions was necessary to ensure that conclusions of the safety assessment were valid.

**Question 23: Given the information before the Panel, including the conclusions of the lead CA's positive opinion (EC-64/At.29-30) and EC SCP opinion, did the additional information provided by the notifier in response to requests by the Dutch CA and by the UK for additional data regarding molecular characterization address outstanding scientific concerns related to potential risks associated with this product (EC-64/At.113 and 119)? Was the molecular characterization provided by the applicant sufficient to enable the Dutch CA and the SCP to conduct a risk assessment and to support the conclusions reached regarding the safety of the product?**

**Dr. Andow**

433. The issue addressed in EC-64/At.104-105, 19 October 1999, was the existence and sequence of backbone DNA. This issue had not been addressed previously for this transgenic fodder beet. The notifier indicated that they would provide information documenting that the backbone DNA was absent, but in the meantime would provide a risk assessment assuming the entire backbone DNA was present (EC-64/105, 19 October 1999). This risk assessment was not available in any of the materials before the Panel. The notifier provided the Dutch CA with the molecular evidence in EC-64/At.113, 23 February 2000.

434. This issue addresses the possible activity of backbone DNA in the plant. If it exists and it becomes active, it could result in unanticipated phenotypes that could lead to adverse effects on human health or the environment.

435. Thus I conclude that the additional information addressed an outstanding scientific concern related to the potential risks associated with this product.

436. The UK CA also addressed the same issue to the notifier, apparently unaware that the issue had been raised by the Dutch CA and addressed already by the notifier. The notifier clarified this in EC-64/At.119, 12 July 2000.

437. The molecular data was included with a summary and transmittal letter from the notifier on 23 February 2000. I will not comment on the sufficiency of these data.

**Monsanto Roundup Ready cotton (RRC1445)**  
**C/ES/97/01 (EC chronology 66)**

**Question 24: Does the information before the Panel, including the full application (EC-66/At.3-12) and the EC 's SCP opinion (EC-66/At.43), provide scientific support for the objections raised by EC member States (EC-66/At.57) concerning the adequacy of the monitoring plan, the antibiotic resistance marker genes, and herbicide residues?**

- (a) **What criteria can be used to judge if the final monitoring plan submitted by Monsanto in January 2003 was complete?**

**Dr. Andow**

Scientific support for objections:

438. This response addresses only issues related to the adequacy of the monitoring plan, and does not address issues related to antibiotic resistance marker genes and herbicide residues.

439. The lead CA reached the following conclusions (EC-66/At.05, Submission of lead CA to COM, 19 Nov 1997, Overall Assessment).

2. From the risk assessment it is concluded that there is no reason to suppose that the harvest and handling of RRC Line 1445 tolerant to glyphosate herbicide, have adverse effects on the environment and human health.

9. The dossier was considered by National Commission on Biosafety. The main aspects considered in the risk assessment were:

- Capacity to survive, establish and disseminate.
- Potential for gene transfer.
- Products of expression of inserted sequences.
- Phenotypic and genetic stability.
- Pathogenicity to other organisms.
- Potential for adverse effects to humans.

10. The National Commission on Biosafety considers that, for the considered uses, there is no significant difference as far as environmental and human health risks related to other cotton.

16. Finally, as far as potential effects for non-target organisms is concerned, CP4 EPSPS protein is broadly present in nature. Therefore organisms which feed with plants and microorganisms are exposed to this protein. Studies with birds fed with RRC line 1445 cottonseed meal have been conducted and no significant difference of this feeding was detected. Moreover, EPSPS protein exist in nature and is considered non-toxic to animal species. On the other hand, cotton is a unique crop that mammals and other species which consume vegetation avoid feeding on the plant due to both gossypol in the plant and the morphology of the plant.

440. These excerpts show that the risk assessment dated 19 November 1997 by the NCB did not consider indirect effects on non-target organisms, long-term or spatial scale effects on non-target species, effects associated with changes in the cropping system or the evolution of resistance in weeds to glyphosate. The NCB does consider whether there are any significant differences between anticipated risks from RRC 1445 compared to conventional cotton. Although the NCB concludes that there are no significant differences (point 10), they do not provide a scientific argument that this conclusion extends to the potential effects not considered in the risk assessment. Because the NCB considered that the risks they assessed were inconsequential, the NCB did not propose any monitoring plan.

441. The SCP provided the following Opinion (EC-66/At.43, 14 July 1998. Opinion of the Scientific Committee on Plants).

6.3.3. Safety to non-target organisms: Exposure of non-target species to seeds can be considered very low, due to the morphology of the boll. Feeding studies with birds (seeds) and mammals (both proteins) indicate very low toxicity of the proteins which also occur ubiquitously in the environment in plants and microorganisms. Field studies on agronomic performance showed equivalent susceptibility of line RRC 1445 and non-modified varieties to diseases and insect pests.

6.3.4. Resistance and tolerance issues: Any selective advantage of cotton line RRC 1445 is restricted to cases where no herbicide other than glyphosate is used on early stages of cotton. Under normal application rates, the introduced glyphosate-tolerance is effective up to the 4-leaf stage only. Other herbicide, cultivation of rotational crops or winter conditions will kill both modified and non-modified plants. Volunteers should be dealt with by standard agricultural practice except that glyphosate should not be used.

442. These excerpts show that they did not consider indirect effects on non-target organisms, long-term or spatial scale effects on non-target species, effects associated with changes in the cropping system or the evolution of resistance in weeds to glyphosate. Because the SCP considered that the risks they assessed were inconsequential, the SCP did not propose any monitoring plan.

443. Sweden commented as follows (EC-66/At.57, 26 April 1999. Consultation of the Committee by Written Procedure).

Sweden has, in different EU contexts and in earlier statements, put forward the view that herbicide tolerant crops should not be placed on the market until the long-term effects of herbicide tolerant crops on the environment have been better analysed. Common principles for evaluation and monitoring of the risks connected to the cultivation of herbicide tolerant crops should be established.

444. Sweden suggests that long-term effects should be studied prior to marketing and that principles for monitoring risks related to the cultivation of herbicide tolerant crops should be established. Sweden is saying that effects associated with changes in the cropping system should be considered both in the assessment and monitoring of risk. The scientific literature at that time indicated that long-term effects frequently elude detection when assessed on short time scales, so one of the only reasonable ways to address these kinds of effects is via monitoring. It does not seem appropriate to require long-term experiments for risk assessment because that could delay the process by a decade, so monitoring is a possible alternative.

445. The United Kingdom commented as follows (EC-66/At.57, 26 April 1999. Consultation of the Committee by Written Procedure).

We would also like to draw to the attention of other Member states where GM cotton might be widely grown, that this cotton line may have a negative impact on biodiversity arising from changes in the way the herbicide tolerant crop is managed.

446. The United Kingdom is drawing attention to spatial scale effects and effects associated with changes in the cropping system. The scientific literature at that time indicated that large-scale effects

frequently elude detection when assessed at smaller spatial scales. One of the reasonable ways to address these kinds of effects is via monitoring.

447. The information in the full application (EC-66/At.3-12) and the EC's SCP opinion (EC-66/At.43) do not provide scientific support for the objections raised by EC member States (EC-66/At.57) concerning the adequacy of the monitoring plan. This is because the scientific information in the full application and the EC-SCP opinion do not address the scientific basis for the objections raised by the EC member states concerning the adequacy of the monitoring plan.

448. The objections of the member States (EC-66/At.57) concerning the adequacy of the monitoring plan raise new scientific issues that were not assessed in the full application (EC-66/At.3-12) or the EC's SCP opinion (EC-66/At.43). There is no monitoring plan proposed in either the full application (EC-66/At.3-12) or the EC's SCP opinion (EC-66/At.43), so the EC member States' objections can only be interpreted that a monitoring plan may be or is necessary.

449. **The objections of the member States (EC-66/At.57) concerning the adequacy of the monitoring plan are scientifically justifiable.** The EC member States raise specific scientific issues that can be addressed in a monitoring plan. However, the necessity of a monitoring plan cannot be determined from these objections.

450. It should also be noted that the objections of the member States (EC-66/At.57) concerning the adequacy of the monitoring plan are not all clearly stated as monitoring issues, and the objections do not provide clear guidance to an applicant for how to fully respond.

451. Excerpt from EC-66/At.64. Letter from Spanish CA to Monsanto requesting additional information regarding the monitoring plan, 1 January 2003.

Plan de seguimiento

Se deberán concretar y desarrollar aquellos aspectos susceptibles de ser sometidos a una vigilancia general, indicando las posibles actuaciones en cada caso.

En este sentido, el Plan de seguimiento deberá contemplar, en su caso, el uso del herbicida Glifosato y sus potenciales efectos a largo plazo (incidencia de malas hierbas, resistencias, etc), así como cualquier otro efecto relacionado con cambios en las prácticas agrícolas convencionales.

452. I believe the Spanish CA is requesting that the monitoring plan address potential effects of large spatial scale (for example, weed incidence, resistance) and in relation to changes in agricultural practice. These echo the comment of the UK (EC-66/At.57) and some of the comment of Sweden (EC-66/At.57).

453. The UK Farm Scale Evaluations (FSE)<sup>65</sup> of GMHT crops published in 2003 indicate that one anticipated effect of GMHT crops is an alteration in weed populations and communities compared to conventional production. The precise alteration probably depends on the herbicides used on the

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<sup>65</sup> Andow 2003, Nature Biotechnology, 21, 1453-1454. Brooks et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1847-1862. Champion et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1801-1818. Firbank 2003, Phil. Trans. R. Soc. Lond. B, 358, 1777-1778. Haughton et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1863-1877. Hawes et al. 2003, Phil. Trans. R. Soc. Lond. B, 1899-1913. Heard et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1819-1832. Heard et al. 2003, Trans. R. Soc. Lond. B, 358, 1833-1846. Roy et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1879-1898. Squire et al. 2003, Phil. Trans. R. Soc. Lond. B, 358, 1779-1799.

GMHT crop and the conventional crop. Nearly all other adverse effects on non-target species would likely follow from these changes in weed populations and communities. Changes to weed populations and communities have not been reported in GMHT cotton on a scale similar to the UK-FSE trials. These studies follow on a concern published in 2000 that GMHT crops could adversely affect skylarks in the UK.<sup>66</sup> Although none of these studies existed at the time of the decisions by the NCB or the SCP, they partially validate some of the hypothetical concerns expressed by the various countries, especially related to spatial and temporal scale.

454. Considerable theory and evidence indicates that another anticipated effect of GMHT crops is the development of resistance to glyphosate in some weed populations.<sup>67</sup> Resistance to glyphosate in a species of morning glory, which is a weed found in GMHT cotton in southeastern US, has been recently reported.<sup>68</sup> However concerns about resistance have been widely recognized for some time before 1998.

455. Potential long-term effects of GMHT cotton have not yet been identified and verified scientifically.

456. The Member States submitted questions to the notifier on 22 February 1999. The notifier submitted materials to the SCP on 6 July 1998. The SCP returned an Opinion on 14 July 1998. The notifier submitted additional information that was circulated on 20 November 1998. After this follow 3 decisions to postpone the decision-making process. Depending on which dates are used, the Member States used either 94 or 231 days to respond to the most recent submission by the notifier. The shorter time frame is reasonable, especially as it spans the winter holiday season when most offices are closed for long periods of time. The longer period of time seems excessive to formulate and provide the responses to the notifier.

Criteria to judge monitoring plan:

457. The specific purpose of monitoring should be clearly stated. This purpose should be linked to the management of some risk. This linkage ties monitoring to risk management, and delimits the monitoring endpoint. Although Annex VII.A of Directive 2001/18/EC provides a statement about the objective of a monitoring plan, this statement is not specific enough as the purpose of any particular monitoring plan. The plan should be more closely linked to actual potential risks, as indicated in Annex VII.C.1-2 of Directive 2001/18/EC.

458. For example, there are several possible purposes for monitoring herbicide resistance<sup>69</sup> in weeds, including:

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<sup>66</sup> Watkinson et al. 2000, *Science*, 289, 1554-1557.

<sup>67</sup> See [www.weedscience.org](http://www.weedscience.org), which lists all occurrences of weeds resistant to a herbicide worldwide, including cases of resistance to glyphosate.

<sup>68</sup> Baucom and Mauricio 2004, *PNAS* 101: 13386-13390.

<sup>69</sup> Here I will not distinguish between resistance and tolerance, and will refer to both as "resistance." Baucom and Mauricio (2004, *PNAS* 101: 13386-13390) distinguish tolerance from resistance, basing the distinction on Painter's (1958, *Annu. Rev. Entomol.* 3: 267-290) framework. They define tolerance as a trait to compensate from herbicide damage and resistance as a trait that prevents herbicide damage. Painter, however, considered both of these to be a part of the concept of resistance, and suggested that resistance is divided into "tolerance", "antibiosis" and "nonpreference." Antibiosis and nonpreference correspond to the narrow definition of "resistance" advanced by Baucom and Mauricio. I adopt the broader definition of resistance stemming from Painter's work.

- Purpose R1. Document/Measure the occurrence of herbicide-resistant weeds. This would provide information prior to any control failures that could be used to alter the use of the herbicide or herbicide-tolerant<sup>70</sup> crop to delay or avoid higher levels of resistance in the weed. Because this occurs before any control failures, it would provide time to develop a reasoned response to the threat. This would be particularly advantageous to prolong the useful life of the herbicide in question, especially if it had replaced herbicides that caused greater damage to the environment.
- Purpose R2. Document/Measure the occurrence of a weed problem that cannot be controlled by the herbicide. This could provide information of localized control failures that could be used to alter the use of the herbicide or herbicide-tolerant crop to delay or avoid higher levels of resistance in the weed. Because some control failures will have occurred, it may be necessary to mobilize a rapid response to prolong the useful life of the herbicide.
- Purpose R3. Document/Measure the widespread occurrence of a weed problem that cannot be controlled by the herbicide. This would provide information of widespread occurrence of the risk, and would be an indication that the risk management practices had failed. One crucial use of monitoring is to determine when a predefined point of failure is reached.

459. Similarly there are several possible purposes for monitoring changes in weed populations and communities, including:

- Purpose W1. In the UK, there has been a concern that GMHT crops would adversely effect the skylark, a desirable species.<sup>71</sup> Skylarks feed on weed seeds, so monitoring of weed populations and communities could be aimed to monitor the abundance of food for skylarks. This approach could be extended for any other non-target species of concern.
- Purpose W2. There is a possibility that "unanticipated" or "unexpected" adverse effects could follow from changes in weed populations and communities. Monitoring these weeds would be a prelude to discovery of unanticipated or unexpected adverse effects.

460. In addition, there are several possible purposes served for monitoring for "unexpected" or "unanticipated" effects, including:

- Purpose U1. Document the geographic and temporal pattern of use of the GMHT crop so that there is a database available that would enable retrospective epidemiological-like investigations should an "unanticipated" or "unexpected" effect be observed.<sup>72</sup>
- Purpose U2. Train personnel who normally visit agricultural fields and natural areas near agricultural fields to be aware of the possibility that agriculture generally and GMHT crops specifically may have "unexpected" or "unanticipated" effects. Inform these people of the occurrence of GMHT crops. Training should include information

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<sup>70</sup> The use of "tolerance" in this context is different from the use of tolerance as defined either by Painter (1958) or Baucom and Mauricio (2004). In actuality, these GMHT (genetically-modified herbicide-tolerant) crops are not damaged by the herbicide, unless the herbicide is applied inappropriately.

<sup>71</sup> Watkinson et al. 2000, *Science*, 289, 1554-1557.

<sup>72</sup> This was recommended by an NRC committee (NRC 2002, *Environmental effects of transgenic plants: The scope and adequacy of regulation*. NAS Press, Wash, DC). It finds resonance in Annex VII.C.4 of Directive 2001/18/EC, but the Annex is not as clear as the recommendations in NRC 2002.

about the potential mechanisms by which such effects could possibly arise. For example, "unanticipated" effects of weed community shifts should receive attention. This training will provide the many eyes needed to notice possible changes in the environment.<sup>73</sup>

- Purpose U3. Document changes in agricultural practices associated with the use of GMHT crops. There is a possibility that "unanticipated" or "unexpected" adverse effects could follow from changes in agricultural practices. Monitoring these practices could be a prelude to discovery of unanticipated or unexpected adverse effects.

461. Clear specification of the purpose of monitoring is probably the most critical step in developing a useful monitoring system.

#### Monitoring must have a sequel

462. The act of monitoring should not be an end in itself. The information gathered during the monitoring activities should be used to make a response. This response should be related to the risk, including activities ranging from those to better estimate the risk to those activities to avoid, mitigate or tolerate the risk.<sup>74</sup> Specification of a response is probably the second most critical step in developing a useful monitoring system. Although Annex VII.C.6 of Directive 2001/18/EC states that a response should be considered, it does not require a response.

#### Action Trigger

463. In addition to a sequel, a monitoring system must have a well-defined action trigger. An action trigger is a criterion or set of criteria, which if met (or exceeded) according to the information from monitoring, would require taking the response actions. Without a clear action trigger, it could become quite difficult to determine when monitoring would precipitate a response.

464. Important logistical issues include: (1) who will monitor, (2) who is responsible for monitoring, (3) who handles and synthesizes the monitoring information, (4) who verifies the quality of the monitoring, information obtained from monitoring and synthesis of this information, (5) to whom are monitoring results reported and at what frequency, (6) how will the monitoring effort be sustained, (7) how can monitoring be conducted in a cost-effective manner. Most, but not all, of these logistical issues are recognized in Annex VII.C.3 and 5 of Directive 2001/18/EC.

465. Important methodological issues include: (1) what 'endpoint' will be monitored, (2) what frequency will monitoring be conducted, (3) what will be the spatial density (grain) of monitoring, (4) can monitoring be stratified by potential risk. A few of these issues are recognized in Annex VII.C.3 of Directive 2001/18/EC. The endpoint of monitoring is what is actually measured and/or estimated by the people conducting the monitoring. For example, for weed resistance it could be the frequency of resistance genes or resistant phenotypes in the weed population; for non-target effects, it could be total weed biomass, production of weed seeds that are normally consumed by skylarks, and so on. Choice of endpoint can enable or restrict possible responses. The frequency of monitoring will depend on several factors, including the anticipated response. If the response involves a series of

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<sup>73</sup> This was recommended by an NRC committee (NRC 2002, Environmental effects of transgenic plants: The scope and adequacy of regulation. NAS Press, Wash, DC). It finds resonance in Annex VII.C.3.2 of Directive 2001/18/EC, but the Annex is not as clear as the recommendations in NRC 2002.

<sup>74</sup> This point has been made emphatically in NRC 2001. Ecological monitoring of genetically modified crops. National Academy Press, Washington, DC. 45pp.

processes that would take several years to complete, it might be appropriate to increase monitoring frequency so that the monitoring process does not introduce additional delays in responding to a potential threat. The grain of monitoring will be depend on several factors, including the expected grain of the effect being monitored. For example, if regional weed resistance is the concern, then monitoring can be done at a regional scale. If weed resistance on farms is the concern, then monitoring will probably need to occur at a finer grain. Weed community shifts might be done on several spatial scales. If the adverse consequences are focused on bird species, regional monitoring may be sufficient. If they are focused on less mobile species, then a finer grain will be necessary. Finally, it will be essential that monitoring is stratified so that it occurs at places and times most likely related to possible risks. Monitoring is costly and monitoring resources must be allocated efficiently. For example, monitoring for unanticipated effects could be stratified according to the geographic distribution of the GMHT crop. Monitoring could occur mostly or only at places where the GMHT crop is used considerably, with a threshold intensity of usage determining the distribution of monitoring effort.

466. Monitoring for unanticipated or unexpected effects. The first step in developing a monitoring system for these possible effects is to specify clearly what effects, if any, are anticipated or expected. Without this clear statement, it will not be possible to determine if any observed effect is unanticipated or unexpected. For example, if no adverse effects are anticipated or expected, then it must be granted that any subsequently observed effect must be considered unanticipated or unexpected.

467. General critique of proposed monitoring plan (EC-66/At.62). (1) Specific purpose of the proposed monitoring plan is to monitor for unanticipated or unexpected effects. It does not address concerns associated with weed resistance or changes in weed populations and communities, as indicated by the EC officials. The purpose of the monitoring for unanticipated or unexpected effects is not entirely clear. Consequently it is difficult to know how the proposed monitoring will enable the identification of any unanticipated or unexpected effects. (2) The proposed response is to conduct a scientific evaluation of the observed potential unanticipated effect to confirm that it is in fact an unanticipated effect.<sup>75</sup> While it is stated that the response should be proportionate to the risk, it is not clear what procedure and standard will be used to determine a proportionate response. Second is unclear who is responsible for conducting the scientific evaluation and how to ensure that this information will be collected in a timely fashion, who will have access to the information, who determines the adequacy of the design of the evaluation and so on. (3) The triggers for initiating a scientific evaluation are not clear. It would be folly to initiate a scientific evaluation of all possible observations of possible adverse effects. How will these be screened to trigger a scientific evaluation? When does the scientific evaluation provide sufficient evidence to trigger remedial action? These issues are not resolved in the present proposed monitoring plan. (4) The logistical issues are better specified than the other components of the proposed plan. Important weaknesses in the proposal include lack of specification for how the seed supply and distribution network will be linked to the monitoring procedures, that key external networks are not committed to monitoring, procedures to verify the quality of monitoring, information from monitoring and synthesis of this information, short time span and long intervals for reporting, and so on. (5) Methodologically, the proposal has several important weaknesses. No endpoint is specified and it is not clear how people who might be monitoring would recognize an unanticipated effect. In this case these people should be informed that any adverse effect must be considered an unanticipated effect, and should be reported. The frequency and grain of these observations is unclear. There seems to be little stratification of effort by potential risk.

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<sup>75</sup> Page 4, Evaluation of potential adverse effects.

**Dr. Squire**

468. The EC's SCP opinion is generally positive about the application, whereas the EC member states raise several objections. The issue of antibiotic resistance was considered in the SCP's opinion (EC-66/At.53) and found not to pose risk, but there is now widespread perception that antibiotic resistance should not be introduced through GMHT products. The basis of objection by some member states is that general effects of HT cropping on farmland habitats are uncertain, and this applies whatever the species or the land area it occupies. However, the context for this biotech product in cotton is very different from the context of those products which are varieties of oilseed rape. Cotton occupies a very small area in Europe and does not present potential problems of the type associated with oilseed rape or even maize or beet (Table 1). This notwithstanding, and as in other instances, unless criteria can be given, from both the proposer and objector as to what is a desirable or acceptable comparator, then progress with the discussion is impossible, as it became in this instance.

469. There appeared to be no monitoring plan in the original proposal, and in the 2003 document, the relevant section indicated that little specific monitoring was necessary because of the low risk. Monitoring the effects on habitat would be feasible but suitable criteria on which to base monitoring have not been proposed or agreed by either side.

**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?**

**Dr. Nutti**

470. Based on the information provided by the applicant in EC-067/At.13-44 and the analysis done by the EC SCP and following the Codex Guidelines, it is clear that the information required was necessary to support a valid safety assessment. The notifier failed to provide information on several fundamental issues concerning safety such as substantial equivalence, toxicity, protein analysis and animal feeding trials.

**Dr. Andow**

Necessity for valid safety assessment:

471. In this answer I will address only the non-target organism part of the question. The transgenic potato produces elevated concentrations of amylopectin by blocking starch synthesis and also expresses nptII. Based on molecular analysis there are multiple copies of the transgene in the plant.

472. EC-67/At.84, 4 February 1999, First request from SCP to Amylogene for clarification:

In conclusion, I would like to inform you that the Committee will only consider at this stage additional data that is of a non-confidential nature. In this respect, I would be obliged if you would include a statement to this effect in the covering letter with any data you may wish to submit in response to the Committee's questions. The

reason for this is that it is not possible at this stage to repeat the notification procedure, which provides for agreement between the notifier and the competent authority on confidentiality (Article 19 of Council Directive 90/220/EEC). However should you consider that certain of the data requested by the SCP is of a confidential nature and that you would prefer not to submit the data without obtaining a confidential status, the SCP will then complete its evaluation on the basis of the available data.<sup>76</sup>

8) No data are provided on the safety of modified crops to non-target organisms. No risk assessment has been made.<sup>77</sup>

### **3.5. Environmental Aspects**

#### **3.5.1. Geographical relevance of data**

Wherever possible data should be provided from field experience in those geographical regions where the GM plant will be grown commercially to reflect relevant meteorological, soil and agronomic conditions. Where data from field studies on other continents are supplied, the notifier should submit a reasoned argument that the data is applicable to Europe. Bridging studies may be particularly useful.

#### **3.5.2. Impact on non-target organisms**

Clear and well-defined risk assessments should be carried out for each of the different functional environmental compartments that are exposed to the GM plant; this will depend on the specific crop and if any parts of it will remain in the environment after harvest. For example, exposure should be estimated to soil organisms and function (e.g. earthworms, micro-organisms, leaf litter breakdown), non-target arthropods (including pollinators, beneficial arthropods), grazing birds and mammals or, if appropriate, the aquatic environment. This risk assessment should take account of where in the plant the inserted genes are expressed and the consequent exposure of non-target organisms. The assessment should also address the fate of the expressed substance in those environmental compartments where they are introduced and may cause exposure of non-target organisms (e.g. in soil after the incorporation of plant material). Data on the comparative susceptibility of the GM plant to pests and diseases compared to the non-modified plants are useful together with observations on agronomic performance during greenhouse and experimental release trials.<sup>78</sup>

473. The SCP indicated that no non-target assessment had been conducted, and provided guidance (3.5) for completing such an assessment. At this time, possible risks could arise from the transgenic plant or processing wastes, including fruit juices and fruit water.

474. EC-67/At.86, 12 July 1999, Draft opinion of SCP

**6.3.3. Safety to Non-Target Organisms:** There are no data on safety of the modified crops to non-target organisms. However, the results of growing trials

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<sup>76</sup> Page 1. All page references in this answer refer to the page number in the relevant pdf file.

<sup>77</sup> Page 3.

<sup>78</sup> Page 9.

suggest neither greater susceptibility nor greater resistance to pests and diseases than non-GM potato lines. In view of this and the equivalent composition of the modified potato line, it is considered that no adverse effects on non-target organisms would be expected from cultivation of the GM high amylopectin potato line EH92-527-1.<sup>79</sup>

475. The SCP draft opinion reverses their previous opinion that there has been no risk assessment for non-target effects. In what follows, I lay out the findings that appear to lead to both the original opinion and the reversal, and evaluate the scientific basis for each.

476. EC-67/At.77 1 April 1999, Amylogene provides SCP working group with the full application as well as additional data. This is copy of EC-67/At.14 7 May 1998, Summary statement of CA of Sweden

#### Environmental effects of the genetically modified potato clone (EH92-527-1)

In laboratory and field trial studies no differences in behaviour of clone EH92-527-1 have been observed compared to its parental variety. The genetically modified clone is not considered to have increased survivability or competitiveness in agricultural or natural environments. The analysis carried out to investigate whether the modified clone displayed any increase or decrease in frost tolerance did not indicate any such changes. The parental variety has a low flowering frequency and thus a low seed production, and with respect to this the modified clone does not display any change. Compared to the parental variety, no data indicate that the genetically modified clone possess any alteration in its potential to spread its genes, neither through sexual crosses not horizontally to sexually in-compatible species. No unexpected (pleiotropic) effects resulting from the modification of the clone in question have been identified. In conclusion, the modified clone does not exhibit any differences in environmental behaviour as compared to the parental variety (cv. Prevalent), and is therefore not considered to pose any risk to the environment.<sup>80</sup>

477. The summary risk assessment statement of the CA of Sweden on 7 May 1998 does not mention specifically any non-target risks.

478. EC-67/At.79 (also EC-67/At.17) Part I B.7<sup>81</sup>, *Potentially significant interactions of the plant with other organisms than plants in the ecosystem where it is normally grown, including information on toxic effects on humans, animals and other organisms.* Here the notification suggests that changes in glycoalkaloids or nitrates may be causes of non-target effects. The extant literature is reviewed for effects of glycoalkaloids and nitrates on organisms. Mammals, birds, insect pests of potato, nematode pests of potato, pathogenic fungi of potato, pathogenic bacteria of potato and infectious viruses of potato are listed. There is only citations of the toxic effects of glycoalkaloids to some of the listed species. No citations to effects of nitrate are mentioned.

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<sup>79</sup> Page 6.

<sup>80</sup> Page 5.

<sup>81</sup> Page 15 ff.

479. EC-67/At.79 (also EC-67/At.17) Part I D.9

9. Potentially significant interaction with other, not modified organisms

In potatoes normally existing toxic and anti-nutritional substances are glycoalkaloids and nitrates. The interaction with other organisms has been described in Part 1, B.7.. In D.7, in Annex 8 and in Part 3 it is shown that the contents of these substances have not been changed as a consequence of the modification. On that account, the modified potato clone, EH92-527-1, does not affect other organisms in another way than what the unmodified recipient cultivar, Prevalent, does.

The modified clone, EH92-527-1, is characterized by two changes compared to the recipient clone. Those are resistance to kanamycin and the lack of amylose. Because of the latter, the content of amylopectin has been increased to >98%.<sup>82</sup>

There are no reasons what so ever to suppose that the changed starch composition (>98% amylopectin) should cause any danger for other non-modified organisms, including humans. There are already plants, in which this trait has occurred spontaneously or by induced mutations, e.g. maize and barley. Further more "amylopectin starch" can be produced from normal potato starch by chemical treatment. No un-desired side-effects have been observed (Annexes 5 and 7).<sup>83</sup>

480. Specifically amylopectin starch occurs in large quantity in the so-called waxy maize varieties and is also found in quantity in some rice, barley and sorghum varieties.

481. EC-67/At.79 (also EC-67/At.17) Part III D

D. THE RISK THAT THE PLANT CAN BE MORE SUSCEPTIBLE TO PREDATORS AND DISEASES.

The establishment and development of plants from seed of the clone EH92-527-1 has been studied in comparative trials in 1995, and in observation trials in 1993, 1994, 1995 and 1996. In all trials it has been shown that the establishment and development of plants are equal to the recipient clone both above and below ground. During the growing season, both the modified potato clone and the recipient clone were exposed to attack by insects and diseases, mainly potato late blight. In order to avoid such damages, treatments were applied according to the requirements of the recipient clone. There were no observations indicating that the transgenic clone is more (or less) susceptible to any pest or disease than the recipient variety. (Annexes 2, 10, 13, 14, 15 and 20.)<sup>84</sup>

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<sup>82</sup> Page 28.

<sup>83</sup> Page 31.

<sup>84</sup> Page 45.

482. EC-67/At.80 (also EC-67/At.17) Part III E

E. THE POSSIBILITIES FOR NEGATIVE ECOLOGICAL AND OTHER EFFECTS WHICH ARE NOT RELATED TO WEED CHARACTERS

Such effects have not been observed during any of the trials. It is not expected that such effects should occur as a consequence of the modifications present in EH92-527-1 (Annexes 2, 10, 13, 14, 15, 20).<sup>85</sup>

483. It should be clear that the relevant information is in Annexes 2, 10, 13, 14, 15, and 20.

- Annex 2. The Swedish Seed Testing and Certification Institute: Statement on variety distinctness
- Annex 10. Observations in practical cultivation of conventional Prevalent and transgenic Prevalent EH92-527-1, 1994-1995, and in laboratory freezing tests 1996/1997
- Annex 13. Report about field trial with transgenic potatoes 1993
- Annex 14. Report about field trials and seed production with transgenic potatoes 1994
- Annex 15. Report about field trials and practical cultivation with transgenic potatoes 1995
- Annex 20. Report about field trials and practical cultivation with transgenic potatoes 1996

484. Annex 2 is missing from the materials. This annex is the Swedish Seed Testing and Certification Institute: Statement on variety distinctness, so is unlikely to have information relevant to biosafety risk.

485. Annex 10 contains detailed information about 1994 and 1995. Annexes 14 and 15 provide only summary information related to potato seed production, and have no information relevant to biosafety. Annex 20 provides only summary information related to potato seed production, and has no information relevant to biosafety. Annex 13 provides detailed information about 1993 results. However, only four clones were untransformed standards and 79 clones were transformed. This study was too small a scale to provide reliable information related to biosafety.

486. EC-67/At.81 (also EC-67/At.27), Annex 10. The notification concludes as follows:

Conclusions of practical cultivation in 1994 and 1995

The potato clone Prevalent 92-527-1 has not differed from conventional Prevalent in the cultivations observed.

- Normal morphology
- Similar reactions to chemicals

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<sup>85</sup> Page 1.

- Similar resistance to diseases
- Similar sensitivity to frost in early summer as well as in fall

Three comparative trials with comparisons of yield and starch contents were made in 1996 with conventional Prevalent and the clone 92-527-1 (Annexes 8 and 20).

Investigations regarding resistance to nematodes, performed at the Agriculture Laboratory in Lyckeby, revealed identical resistance to *Globodera rostochiensis*, pathotype Ro1Ro4, in both clones.<sup>86</sup>

487. I will show that the conclusion that the clones are similar in resistance to diseases has not been scientifically demonstrated.

488. EC-67/At.81 (also EC-67/At.27), Annex 10. Summary of data. Five plantings are reported, two in 1994 and 3 in 1995. At the 1994 Skara planting, there were bad growth conditions and yield was low and seed quality was bad. Fields were treated with fungicide and no late blight occurred. At the 1994 Norra Sunderbyn planting, growing conditions were good, but there was no late blight in the field. At the 1995 Sofielund planting, growing conditions, plant development and yield were comparatively bad, late blight was similar between transgenic and non-transformed varieties, and there were no attacks of pests or parasites. At the 1995 Habo planting, soil structure was damaged, yield was low and late blight was controlled by normal fungicide applications. At the 1995 Norra Sunderbyn planting, growing conditions were good, but no attack by late blight or other diseases were observed.

489. To show that the clones are similar in resistance to diseases, the diseases must be present and the plants must be healthy. Of the five plantings with sufficient information to evaluate (paragraph 488), none meet these two conditions. If we consider only those plantings with disease present, disregarding the quality of the plants, one planting (1995 Sofielund) would suggest that late blight resistance was similar.

490. It can be concluded that there is no evidence in the written record to support the statement in the draft SCP opinion (EC-67/At.86) that "the results of the growing trials suggest neither greater susceptibility nor greater resistance to pests and diseases than non-GM potato lines." Indeed, even if the statement is restricted to refer only to late blight, it is not supported by the evidence. It is possible that the notifier presented information verbally to the SCP on this issue that is not recorded in the written record, but in this case, the SCP should have mentioned this.

491. Thus it can be argued that **a request for additional non-target information was scientifically justified in 1999 and remains justifiable even today.**

492. However, it is useful to consider if there is any evidence that points to the possible existence of non-target effects. The notification identifies two possible routes by which non-target effects could result: changes in glycoalkaloids or nitrates. Neither of these is likely for amylopectin potatoes, because as detailed in the responses to questions in EC-67/At.83, the amylopectin potatoes do not have different concentrations of nitrate or glycoalkaloids compared to non-transformed potatoes. Two other lines of argument can be considered. First, because amylopectin occurs in other crops, have there been any observable adverse effects from growing these crops? Second, is there any route by which amylopectin or nptII directly could cause an adverse effect? Issues related to nptII are

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<sup>86</sup> Page 8.

extensively covered in the notification, and concerning direct non-target effects, the notification appears to present a strong case that such effects are likely to be small or absent. In the paragraphs below I will take each of these other points in order.

493. Other crops. Using Agricola and CAB Abstracts, I found 1213 references to waxy maize/corn during the past 25 years. I looked at 250 of them and found seven references<sup>87</sup> to the effects of waxy corn on farm animals. Waxy corn does effect animal digestion, but the effects may be possibly beneficial in some animals. There were no studies published on effects on other organisms. This provides some evidence that amylopectin potatoes may have little adverse effect on mammals.

494. EC-67/At.83. Effects on nematodes. The notification reports a summary of an experiment on cyst nematodes. The conclusion was that the transgenic potato had no effect on the nematodes tested. The full data are not included in the notification, and there was no request for the data by the CA or Sweden or the SCP. Without the full data, it is not possible to evaluate the scientific merit of the conclusion.

Reply to questions forwarded by the Austrian Competent Authority.

Your question regarding experimental tests with non-target organisms, in order to verify our statement that the clone cannot be supposed to cause any danger to other non-modified organisms:

As we mentioned during the technical meeting in Brussels on July 23, no such tests have been performed. We are still convinced that the transformed clone cannot be supposed to cause such harm. During growing of the clone we have not been able to note any different reactions in the interaction between the potato plants and the insects and nematodes mentioned. The only analysis made confirms that the transgenic clone carries the same resistance to cyst nematodes (Ro1,Ro4) as does the recipient variety. It should also be mentioned that one goal for potato breeders working with conventional methods (not gene technology) is to introduce resistance to parasitic organisms like beetles and aphids.<sup>88</sup>

495. EC-67/At.83. Fruit water as a possible source of risk. There appears to be little environmental risk associated with fruit water.

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<sup>87</sup> Snow,-J.L.; Stein,-H.H.; Ku,-P.K.; Trottier,-N.L. 2004. Amino acid digestibility and nitrogen utilization of high oil, high lysine, and waxy maize fed to growing pigs. *Animal feed science and technology* 113: 113-126. Akay,-V.; Jackson,-J.A.-Jr.; Harmon,-D.L. 2002. NutriDense and waxy corn hybrids: effects on site and extent of disappearance of nutrients in sheep. *J-anim-sci.* 80: 1335-1343. Many factors examined, generally few significant effects, but some differences reported. Akay,-V.; Jackson,-J.A.-Jr. 2001. Effects of NutriDense and waxy corn hybrids on the rumen fermentation, digestibility and lactational performance of dairy cows. *J-dairy-sci.* 84: 1698-1706. Many factors examined, generally few significant effects, but some differences reported. Dado,-R.G. 1999. Nutritional benefits of specialty corn grain hybrids in dairy diets. *J-anim-sci.* 77 (suppl.2): 197-207. Review. Variable effects reported. Reid,-C.A.; Hillman,-K. 1999. The effects of retrogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. *Anim-sci.* 68 (pt.3): 503-510. Potentially positive effects on young pigs. Kleesen,-B.; Stoof,-G.; Proll,-J.; Schmiedl,-D.; Noack,-J.; Blaut,-M. 1997. Feeding resistant starch affects fecal and cecal microflora and short-chain fatty acids in rats. *J-anim-sci.* 75: 2453-2462. Potentially detrimental effects on rats. Schroeder,-J.-W. (Jerome Wilfred); Park,-C.-S.; Marx,-George-Donald. 199-. Waxy corn as a replacement for dent corn in diets of lactating holstein cows: final summary. Fargo, N.D.: Animal and Range Sciences, North Dakota State University, 14, [8] leaves: ill.

<sup>88</sup> Page 9.

It is necessary to specify the composition of potato processing effluent, count plant cells and provide information about the health and environmental safety of this effluent, in particular for soil bacteria flora, and the process used.

Objection raised by the CA of France.

We assume that the objection concerns the "fruit water" which is spread over fields as a fertilizer.

The fruit water consists of water that is added to the process for washing of the potatoes and for washing of the starch, and a small proportion of fruit juice that is not added to the pulp.

The fruit water contains small amounts of potato skin and flesh residuals, mainly fibres and cells, and water soluble salts of potato constituents.

Consequently, nothing is added to the environment that was not present in the potatoes, and as the water content of the fruit water is > 98%, the amounts of potato material is very small.

It should be noted that fruit water from the starch industry has been used as fertilizers for more than 10 years in Sweden, without causing any problems.

The only constituent of the fruit water that could be a concern is the presence of the nptII gene causing resistance to aminoglycosides (antibiotics). In Part I, Section D, paragraph 9; Part 3, Section C; Annex 1 and Annex 22 of the notification, and in attachment 8 of this document it has been shown that this cannot possibly be assumed to cause any harmful effects by adding antibiotic resistance of any significance to the soil bacteria population.<sup>89</sup>

496. EC-67/At.83. Vitamin C. Amylopectin potato has higher Vitamin C content than the untransformed clones, although the concentration remains within the normal range of variation for potato. Even though it is in the normal range, it is elevated, and could possibly cause a risk. While I consider the following scenario unlikely, there is a possible risk pathway. Holometabolous insects require enough Vitamin C to complete pupation. If pupation is limited for any insect on potato, it could have a higher survival rate on the amylopectin varieties.

- The higher content of vitamin C indicated in the notification is confirmed. The difference between EH92-527-1 and Prevalent is even more pronounced.<sup>90</sup>
- In both years the content of vitamin C was significantly higher in the transformed clone than in the non-transformed variety. This is explained in attachment xx, vitamin C being involved in the sugar and carbohydrate synthesis of the plant. The higher content of vitamin C is not considered to have any impact on animal health or on the environment. It should also be observed that the vitamin C contents measured are within the range of natural variation of the recipient clone (table 9 of the notification). Please note that the values reported in annex 8 of the notification and attachment 1 of this document are expressed as mg/100 g dry matter, while the

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<sup>89</sup> Page 57.

<sup>90</sup> Page 24.

corresponding values of table 9 of the notification are expressed as mg/100 g fresh weight.<sup>91</sup>

Exhaustive comments on the observed significant differences in Vit.C, dry matter, fructose, glucose and sacharose content, associated with the deliberate modification of the starch synthesis.

Objection raised by the CA of Italy

EH92-527-1 produces less starch than the mother variety Prevalent. This is probably because of its inability to synthesise amylose and not using the available sugars to synthesise amylopectin instead. During tuber starch accumulation the tuber is a sink for sugars from the photosynthesis. If those sugars are not incorporated into starch it might result in the higher content of mono- and disaccharides in EH92-527-1. Mutations that affect starch synthesis often result in increased levels of sugars. A mutation with great effects on starch synthesis is where ADP-glucose pyrophosphorylase is affected. This enzyme gives the first committed step in starch synthesis and antisense inhibition of the gene results in potato tubers with decreased dry matter, decreased starch content and increased accumulation of soluble sugars (Müller-Röber et. al.). The modification is not the same as in the present Notification but points in the same direction. A decreased ability to form starch results in increased levels of soluble sugars.

When it comes to Vitamin C, another high amylopectin potato clone is currently being investigated. This new transgenic clone has the same levels of Vitamin C as EH92-527-1. It is to be noted that the content of Vitamin C in EH92-527-1 is within the levels normally observed in potato. A possible explanation is that the higher Vitamin C content is an associate effect of elevated levels of mono- and disaccharides since ascorbic acid is most probably synthesized from simple sugars in a few metabolic steps in plants. In a recent publication (Wheeler et. al.) the pathway leading to Vitamin C in plants was investigated. In the pathway presented, glucose and fructose are starting materials for the biosynthesis of Vitamin C. Therefore an increase of substrate levels might very well result in the significant increase of Vitamin C observed in the present Notification.<sup>92</sup>

497. The transformed amylopectin potato has several transgene loci. Although most of the issues now appear to be resolved, the discussion on molecular characterization implied that the plant might have been producing an unidentified protein from one of these other transgene loci. If such production had occurred, it could lead to non-target risks.

498. From the alternative perspective, it is difficult to believe that amylopectin potato would cause a significant adverse effect on non-target species. Aside from the adverse effects of growing potato, it is not clear what kind of change to potatoes would result in a significantly greater adverse effect. Moreover, if any adverse effects do become manifest, it would be possible to withdraw potato from the market and expect that the transgenic potato would rather quickly die out.

499. In summary, the SCP was justified in asking for a non-target risk assessment on 4 February 1999. In light of the data in front of it at the time, it was not justified in concluding on 12 July 1999

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<sup>91</sup> Page 28.

<sup>92</sup> Page 62.

that amylopectin potato would have no adverse effects on non-target organisms. The scientific data provide no evidence that there is an adverse effect, but the data are not strong. Hence the scientific data and scientific arguments can divide on whether there is a necessity for additional non-target risk assessment. On the one hand, some could argue credibly that adverse effects on non-target organisms are unlikely, and no additional assessment is needed. Others could argue credibly that adverse effects are possible, requiring data to address the few loose ends identified above. In no case, however, could it be credibly argued that adverse non-target effects are likely to or in fact do occur, and therefore an extensive non-target risk assessment is NOT necessary.

500. The SCP requested information from the notifier on 4 February 1999. The notifier provided the full notification on 4 January 1999. The 31 days that elapsed is a reasonable, if not rapid response time.

**Dr. Healy**

Note: the question specifies the Scientific Committee for Food, however there is no mention of this committee in any of the documents, the numbered attachments all refer to information requested by the Scientific Committee on Plants (SCP). I have answered this question in relation to the information requested by the SCP.

501. **Response:** Following receipt of the notification from Amylogene seeking approval for Amylogene starch potato clone EH92-527-1 in August 1996, the lead CA (Sweden) expressed a favourable opinion in May 1998. The dossier was then sent to the European Commission and distributed to other Member States for comment. A number of comments and requests for further information were received from various Member States. In September 1998 the EC requested the SCP to consider whether there was any reason to believe that the placing on the market of the starch potato clone EH92-527-1 would be likely to cause any adverse effects on human health and the environment.

502. The dossier and additional information were supplied to the SCP in January 1999. Between February 1999 and November 2001, the SCP made nine further requests to Amylogene for additional information or clarification of information.

503. **Background:** Potato clone EH92-527-1 contains two novel genes, an antisense Granule Bound Starch Synthase gene (gbss), and a marker gene, the nptII gene. The nptII gene, which is widespread in nature, is widely used in GM plants and is well characterised. The gbss gene was isolated from the potato and was reintroduced into the potato in the antisense orientation to silence the endogenous gbss gene, which is involved in the synthesis of amylose. This results in the production of starch with high amylopectin content. No protein is produced from this introduced gene.

504. The potato clone EH92-527-1 was generated primarily to produce starch for industrial purposes. The by-products of this process, potato pulp, potato juice and potato water, were intended to be used for animal feed and irrigation purposes. No products derived from potato clone EH92-527-1 were intended for use as human food.

505. Molecular characterisation:

4 February 1999 (EC-67/At. 84): the SCP requested further evidence that the plasmid 'backbone' DNA was not incorporated into the genomic DNA:

**More convincing evidence is required that specific elements of "backbone" DNA are or are not incorporated**

**(A) The dossier lacks a detailed map or table describing the DNA backbone outside T-DNA borders. One or other should be provided. Origins of all open reading frames and control elements should be given and a risk assessment provided for each. The notifiers should clarify the antibiotic specificity of the nptII gene incorporated into the backbone of the binary vector.**

**(B) There are some confusing and inconclusive PCR analyses to demonstrate absence of vector sequences flanking the border regions. Appropriate controls are lacking (i.e. positive PCR's are carried out on plasmid DNAs instead of control genomic DNAs or mixtures of genomic and plasmid DNA). The use of overlapping probes in Southern analysis is preferable.**

**(C) Southern blots have been used to determine integration of genes/gene fragments outside left and right T-DNA borders. A cocktail of probes has been used. However, there is no positive control to demonstrate that all elements of the probe are working.**

**(D) Also related to Southern analysis, only 2 probes have been used to determine copy number. No probes have been used covering the nos gene for example.**

506. **Response:** The data in the original dossier concerning the molecular characterisation of potato clone EH92-527-1 included Southern blots of genomic DNA from both the transgenic potato and its parental line, using insert and backbone specific probes. Positive and negative controls were also performed. PCR analysis of the junction regions between the insert and the genomic region indicated that no large fragments of introduced DNA were present at these junctions. However, no sequence data were provided.

507. Southern analysis was originally conducted using a cocktail of backbone specific probes, although this does not conclusively show the absence of plasmid backbone sequence. Evidence was provided that the company intended to supply conclusive data showing no plasmid backbone sequence is present in potato clone EH92-527-1 and DNA sequence of the junction regions (EC-67/At. 83). However, it is not clear from the documents provided (Exhibit EC-67) whether this data was actually provided to the EC or the SCP. This information, together with the data in the dossier provided to the SCP would have made the requests at A), B) and C) unnecessary.

508. A detailed description of the DNA backbone outside of the T-DNA border is not warranted (part A) if sufficient evidence is provided to show that no backbone DNA is present in the DNA of the GM potato.

509. The optimal number of probes to be used in Southern hybridisation is determined on a case-by-case basis (part D). Potato clone EH92-527-1 contains two additional genes the gbss gene and the nptII gene, and therefore as a minimum it would be reasonable to use probes to detect each of these genes. The company used probes specific for the gbss gene and its promoter region. Due to the presence of an endogenous gbss gene, this experimental approach was less than optimal, however, the use of the non-transgenic potato DNA as a negative control allowed identification of the endogenous gene. Although the initial selection procedures confirm the presence of the nptII gene, probes detecting the presence and copy number of this gene in potato clone EH92-527-1 would be required.

510. Toxicity and animal feeding trials

4 February 1999 (EC-67/At. 84): the SCP requested information on the use of potato pulp for animal feeding in different MS, including information on target animals. The SCP also requested justification for the absence of a feeding trial using the GM potato:

**The notifier should provide information on possible application of pulp for animal feeding in different EU Member States. Target animals etc.**

**The notifier should justify the absence of any toxicological data involving relevant animal feeding trials.**

511. **Response:** Potato pulp has a long history of safe use as stock feed. The pulp of the non-transgenic parental line had been used in Sweden as cattle feed since 1988 without known harmful or negative effects. Evidence was provided that the nutritional composition of tubers from potato clone EH92-527-1 was similar to that of the parental line and within the normal reference ranges for commercial potatoes, except for those intended differences related to starch composition. The Swedish Board of Agriculture notified its approval of the transgenic potato for placing on the market in May 1998.

512. The starch present in the pulp from EH92-527-1 differs from common potato starch (composed of amylose and amylopectin) only by lack of the amylose component. The company provided data and estimates of consumption rates in cattle of potato pulp. As amylose usually makes up 20-25% of the total starch in potatoes, if pulp from potato clone EH92-527-1 were to be used, cattle would consume no amylose and more amylopectin per day.

513. Other agricultural crops, such as waxy maize, contain starch with similar levels of amylopectin to potato clone EH92-527-1. Rice, barley and sorghum also contain high levels of amylopectin. Furthermore, the potato pulp used as stockfeed is a by-product of the starch industry and would therefore be unlikely to contain high levels of starch.

514. Given the compositional analyses of the transgenic potato, the similarity in starch composition with other plant derived animal feeds, and the absence of starch remaining in the potato pulp, feeding studies using potato clone EH92-527-1 in various livestock species are not necessary to support a valid safety assessment.

9 March 2000 (EC-67/At. 99): in light of the presence of an ORF in potato clone EH92-527-1 (ORF4, discussed below), the SCP requested an animal feeding study:

**Evidence should be provided regarding the safety of the material for animal feeding since part of the by-products will be used for animal feeding. This is of relevance given the presence of a number of ORFs which show significant homologies to known coding regions. The safety may be assessed by performance of a 90 day feeding trial with laboratory animals administered diets with different levels of potato pulp (concentrated).**

515. **Response:** Further molecular characterization data identified a putative open reading frame (ORF, designated as ORF4) in the transgenic potato. Although transcription was detected, there is no evidence that ORF4 is translated into a protein. Potato tubers are predominantly composed of carbohydrate with only minimal amounts of protein. In this case, the transgenic potato has been shown to be compositionally equivalent to conventional varieties, except for the intended difference

relating to the starch component. Feeding studies using target livestock species will not add substantially to the safety assessment because low dietary exposures to potato proteins would not yield meaningful results that could be validly interpreted.

516. Protein analysis

4 February 1999 (EC-67/At. 84): the SCP requested information on the expression of any novel proteins in potato clone EH92-527-1:

**In addition to the requirement for conclusive Southern blots for components of backbone DNA the Committee would prefer to see Northern/Western analysis where appropriate. For example, the expression of the *nptII* gene and other open reading frames incorporated into the GM plant.**

517. **Response:** Western analysis showing the lack of a gbss gene product in potato clone EH92-527-1 was provided. These results demonstrate the effectiveness of the genetic modification. Given that the introduced antisense gbss gene has a silencing function, the results of Northern analyses would add little to the assessment of safety of the transgenic potato. Messenger RNA from the endogenous gbss gene would normally be detected and no additional safety concerns would be raised by the presence of the antisense gbss transcripts.

518. However, the dossier contained no data on the expression of the *nptII* gene. As the only novel gene expected to be expressed in the transgenic potato, data and information on the expression of *nptII* would be required. Rather than Northern analysis, antibody based techniques (such as ELISA) would be sufficient to determine the levels of APH(3')II expressed in the tubers.

519. Several other ORFs are present in the inserted DNA. In the first instance, bioinformatic analysis of any hypothetical polypeptides would be an appropriate means of assessing the significance of these in the potato. Depending on these results and the presence of any putative regulatory elements, Northern analysis may be justified. This analysis would be particularly important where significant homology with known protein toxins or allergens was identified, or where information from other parts of the safety assessment indicated a potential safety issue.

520. **ORF4:** Over the period from June 1999 to November 2001, the SCP required further information on an identified ORF in the inserted DNA, designated ORF4. ORF4 lies between the 3' end of the *nptII* gene and the *nospA* element (the polyadenylation signal) and is not in the same reading frame as *nptII*. ORF4 was identified as having some homology to the bleomycin resistance protein of Tn5 (*E. coli*) and the ornithine cyclodeaminase protein (from *Agrobacterium tumefaciens*).

3 June 1999 (EC-67/At. 85): the SCP requested the following information from Amylogene:

**ORF analysis indicates homologies within ORF4 to the bleomycin resistance protein of Tn5. Bleomycin is a chemotherapeutic used in cancer treatment. The SCP wishes to know the significance of the presence of this ORF with respect to the expression of a protein and to the possibilities of transfer of a functional bleomycin resistance gene to non-target organisms. This could be determined by Northern blot analysis of potato leaves and tubers.**

521. **Response:** The safety assessment includes an evaluation of the significance of unintended translation products arising from the genetic modification on human health and safety. Evidence would be required to ascertain whether expression products could arise from ORF4, and including an evaluation of the potential toxicity and allergenicity of these products. Northern blot analysis would

normally be expected to detect transcription of functional genes, but due to a lack of sensitivity, may not detect low abundance messages.

522. The exquisitely sensitive technique of reverse-transcriptase PCR (RT-PCR) was performed and transcription across the nptII gene into ORF4 was detected. However, this result does not demonstrate that translation of ORF4 would occur. As ORF4 lies between the nptII gene and its polyadenylation signal, a transcript corresponding to this region of DNA may be expected. Furthermore, the presence of a stop codon immediately following the nptII gene strongly suggests that translation stops at the 3' terminus of this gene. In addition, ORF4 is not in the same codon reading frame as the nptII gene and would therefore require additional regulatory signals for translation.

523. Theoretical evaluation of the safety of any translation product from ORF4 is required in the assessment. Only the first 50 potential amino acids of ORF4 are homologous to the bleomycin resistance gene (total of 126 amino acids). This peptide has been shown to have no bleomycin resistance activity. Therefore, in the unlikely event of transfer of this putative coding region to micro-organisms, bleomycin resistance would not be transferred.

524. Additional bioinformatic analysis would determine if ORF4 has any homology with any known protein toxins or allergens. However, given that there are no indications that ORF4 produces a protein and that exposure to any proteins from potato clone EH92-527-1 by animals is likely to be extremely low, the transcription of ORF4 does not raise any safety concerns.

525. Therefore, the additional information requested by the SCP on the potential expression of ORF4 would contribute to the validity of the safety assessment. It should be noted that in seeking information from the company, the SCP could have anticipated the need for additional molecular and expression analysis data concerning ORF4. For example, sequence analysis of ORF4 to detect any homology with known protein toxins or allergens was indicated on identification of ORF4.

9 March 2000 (EC-67/At. 99): the SCP requested Amylogene to conduct bioinformatic analysis of the amino acid sequence of ORF4 to provide information on the possible allergenicity of any polypeptide that might be produced:

**The SCP accepts the notifier's evidence that no functional bleomycin resistance protein is produced from ORF4. However, the SCP asks the company to provide information on the possible allergenicity of any polypeptide that could be produced. The SCP accepts that this can be completed by comparing sequence homologies using relevant databases.**

526. **Response:** It is noted above that these data are indicated in this case to assess the safety of potato clone EH92-527-1 and therefore in my opinion were required as part of the dossier.

20 July 2000 (EC-67/At. 100): the SCP requested Amylogene to repeat this analysis to search for similarities within ORF4 without any reference to the word "allerg" in the query:

**Swiss prot databases have been queried for entries with sequence ID's combined with any word beginning with "allerg" in any field. For completeness the SCP asks the notifier for a full search for, and list of, similarities within ORF4 without reference to the word "allerg" in the query.**

527. **Response:** This second search is unlikely to add any new information to the assessment of ORF4 for potential allergenicity. It may, however, identify sequence similarity with known toxins in

the database, as it appears that this specific information had not been supplied to the SCP at this stage. This analysis would be consistent with a thorough safety assessment.

20 July 2000 (EC-67/At. 100): Amylogene was requested to produce antibodies to the ORF4 polypeptide so as to be able to test for expression in potato clone EH92-527-1.

**The SCP thanks the notifier for their efforts in attempting to prepare recombinant polypeptides using ORF4 and expression vectors. The notifier is requested to attempt antibody production using synthetic polypeptides raised to immunogenic sequences within ORF 4. This should include sequences in ORF4 similar to MP12 AMBAR. Several peptides should be prepared from sequences spanning components of ORF4 to improve the chance of success. Antibodies could be raised in mice, in addition to rabbits, to accelerate the process of risk assessment.**

528. **Response:** Detailed bioinformatic analyses did not indicate homology of the putative ORF4 amino acid sequence with any known protein toxins or allergens. Furthermore, as stated above, there is no evidence that ORF4 is translated. Based on the totality of the information on potato clone EH92-527-1 and its intended uses, sufficient information is available to support the conclusions of the safety assessment. Further analysis including Western blotting requiring ORF4 specific antibodies is not justified.

Between 20 July 2000 and 8 November 2001, the SCP made a number of requests to Amylogene, concerning the Western analysis of potato clone EH92-527-1 in relation to expression of ORF4. These questions are listed below:

**1 February 2001 (EC-67 Attachment 102)**

**It is necessary to see an alignment of the amino acid sequences used to generate the synthetic peptides and the original nucleotide sequence.**

**In Western blots, figure 1(b), the immunoresponse of the GM leaf protein in lane 12 is very different from that of the parent line. In lane 12 two major bands are missing ca 40 and 55kDa, yet a band appears to occur between the 40 and 55kDa proteins (ca52kDa?). This band appears to be present in lanes 4 and 5 but not in lanes 7, 8, 9, and 11. This implies that expression of ORF4 results in the formation of a polypeptide much greater than 17.5 kDa. lane 12 implies that this polypeptide and ca 52kDa, resulting from expression of ORF4 in *E. coli* is in fact presenting the GM line. The interpretation of these data by the notifier is requested.**

**Proteins in lane 12 do not appear well resolved compared with lanes containing non GM-derived leaf protein. There is a need to repeat the blots with adequate replication and with coumassie stained gels shown in parallel. The notifiers should consider Western blots with tubers as well as leaves.**

**Western blot the purified ORF4 polypeptide from *E. coli* not a crude *E. coli* extract.**

**14 March 2001 (EC-67 Attachment 104)**

**Statements that recombinant ORF4 is a 17.5 kDa protein whereas in the GM plant the polypeptide would be 14.4 kDa. Fig 5 indicates that the recombinant protein is >25 kDa?**

In Fig 4c a cross reacting band appears at 48 kDa in all lanes with leaf proteins from the GM line (lanes 3-9, lane 12) but not in the non GM parent (lane 11). This band cannot represent *E. coli* protein as its intensity does not change with dilution of the partially purified ORF4. This band was commented upon in an earlier question to the notifier (see Fig 1). We also note that the Western blot pattern for Fig 1 is significantly different from Fig 4 (with the same samples?)

With respect to dilutions of ORF4 used to "spike" potato protein samples, there appears to be very distinct differences in sensitivities for gels in Figs 4 b and d in particular then the presence of any ORF4 in tubers may be missed?

The coomassie stained gel shown in Fig 4e (which represents the same samples in each lane shown in Fig 4c) indicates that the polypeptide profile in lane 11 (the non GM parent) is very different from the profiles for the GM line. Does this indicate a lack of substantial equivalence?

The SCP would also like to discuss the numbers and nature of replicate leaf and tuber samples chosen and the nature of any additional work that might be needed to formulate a final risk assessment.

3 April 2001 (EC-67 Attachment 105)

The notifier should provide details on the construct used to express ORF4 in *E. coli*

Since there is still a question over the ca 48 kDa protein it will be important for the notifier to prove their hypothesis that the band is non specific and represents an endogenous, naturally occurring potato protein. This will mean carrying out Western blots with samples from equivalent leaf positions using plants of equivalent age and physiological status. Leaves should be used from several non GM and GM plants and experiments for replication purposes. The notifier could also include comparisons with other antisense GBSS potato GM lines that company might hold to emphasise their argument. Replicated tuber Western blots would also be very beneficial. It will be important that the sensitivities of the various Westerns are as similar as possible so that any relevant immunological cross reactions are not missed. Spiking some lanes with ORF4 protein, as done previously, appears to be a good way to approach this. In addition to the Western blots, parallel SDS-PAGE and coomassie stained gels of leaves and tubers will also allow the SCP to determine equivalence of the samples selected in terms of overall polypeptide profile.

8 November 2001 (EC-67 Attachment 106)

The SCP requires that all data proceed in response to questions to notifiers to be of a standard acceptable for publication in a well-regarded peer reviewed journal.

The quality of Western blots provided to date and using antibodies raised against ORF sequence is not considered adequate. More specifically:

The sensitivity of the detection system appears highly variable from blot to blot for known amounts of ORF4 protein. In SDS-PAGE and coomassie stained gels control ORF protein is visible in some cases but absent in others. This raised questions on the overall reliability of data presented.

**It may be pertinent for the notifier to consider sub-contracting the Western blotting to a third party.**

**The notifier should seriously consider additional Western blots using antibodies raised specifically against the nptII protein. This will add weight to arguments that the polycistronic RNA associated with ORF4 does not give rise to a fusion protein (molecular mass greater than that of nptII protein alone).**

529. **Response:** The totality of the data and analysis of ORF4 already supplied to the SCP supports the validity of the safety assessment without the need for Western blot analysis of ORF4.

530. APH(3')II (nptII gene product)

4 February 1999 (EC-67 Attachment 84 Question 6): the SCP requested that the levels of APH(3')II protein be provided for tubers and plant tops:

**The concentrations of NPTII protein should be provided for tubers and plant tops (haulms).**

531. **Response:** A number of countries, including Canada and Australia would usually require this information for the safety assessment of a GM plant intended for use as human food. However, the guidance document for preparing dossiers (SCP, 1999), states that this information may be required if a potential risk is identified.

532. The nptII gene was used as a selectable marker gene in development of the potato clone EH92-527-1 and is expressed in the transgenic potato. It is generally accepted that information on expression levels of novel proteins in GM plants are required for the safety assessment.

533. The nptII gene has been used often as a marker gene in transgenic plants. From previous evaluations it has been established that the nptII gene and resistance to kanamycin and neomycin are ubiquitous in nature and that neither the gene nor its protein product pose a risk to human, animal or environmental health when used in GM plants. It is therefore unlikely that information on the expression levels of nptII in potato clone EH92-527-1 would adversely influence the safety assessment. However, expression levels would be of interest to determine that patterns of expression in the transgenic potato are as expected. Therefore this request is warranted to ensure the safety assessment is comprehensive and consistent with other regulatory bodies internationally.

3 June 1999 (EC-67/at. 85): 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) was requested by the SCP:

***In vitro* data on the survival of nptII protein, preferably supplied as leaf tissue in the presence of rumen micro-organisms should be provided. Should ORF4 be expressed and produce a protein then the extent of its digestion in the presence of rumen micro-organisms should also be determined.**

534. **Response:** There is concern that horizontal transfer of antibiotic resistance genes from transgenic plants to gut micro-organisms may contribute to increased resistance against clinically important antibiotics. The potential impact this may have on human health is assessed on a case-by-case basis.

535. However, as mentioned previously, the nptII gene is already ubiquitous in nature and has been considered by the World Health Organization to pose no additional risk to human or environmental health when used in GM plants (WHO, 1993). Although ORF4 has some homology to a bleomycin resistance gene, is not functional and therefore would not confer resistance and would pose no additional risk if it were to be transferred to micro-organisms in the gut.

536. Effects on non-target organisms

4 February 1999 (EC-67/At. 84): the SCP queried why no data were provided on the safety of modified crops to non-target organisms:

**No data are provided on the safety of modified crops to non-target organisms. No risk assessment has been made.**

537. **Response:** The nature of this transgenic crop is not such that effects on target organisms would be anticipated. Compositional analysis did not identify any changes in the nutrient or antinutritional profile of the GM potatoes that would suggest concern for non-target organisms. Agricultural practises for this crop are identical to conventional starch potatoes. Amylogene did not observe any unusual interaction between the transgenic potato plants non-target organisms, such as insects, during field trials of this crop.

538. Given this information, further data on the effects of the transgenic potato on non-target organisms are not warranted to support a valid safety assessment.

539. Bleomycin resistance

3 June 1999 (EC-67/At. 85): the SCP requested clarification on the potential of ORF4 to provide resistance to bleomycin and the possibility of transfer of a functional bleomycin resistance gene to non-target organisms:

**ORF analysis indicates homologies within ORF4 to the bleomycin resistance protein of Tn5. Bleomycin is a chemotherapeutic used in cancer treatment. The SCP wishes to know the significance of the presence of this ORF... with respect to possibilities of transfer of a functional bleomycin resistance gene to non-target organism.**

540. **Response:** The guidance document for the preparation of dossiers (SCP, 1999) and the Codex Guideline for the Conduct of Food Safety Assessments of Foods Derived from Recombinant DNA Plants (CAC, 2003) recommend that the safety assessment of GM plants should take into account the potential impact of horizontal DNA transfer between the plant or plant components and micro-organisms.

541. Therefore, it important to determine whether the potential ORF4 polypeptide would confer bleomycin resistance and what impact this would have on human health and the environment if a functional bleomycin gene were to be transferred to other organisms, for example micro-organisms.

542. Substantial equivalence

4 February 1999 ( EC-67/Att. 84): the SCP requested additional statistical analysis of the compositional data:

**Evidence of substantial equivalence: data from 1996 and 1997 should be treated for statistical purposes as a single data set and, ideally, strengthened by including data from the 1998 season. To fully justify co-variant analysis and conclusions derived from it, the interdependence of yield and composition should be addressed for two to three growing Seasons**

543. **Response:** The SCP guidance document for the preparation of dossiers (SCP, 1999) indicates that compositional analyses should be based on samples from at least two growing seasons, with the growing sites being diverse in number and location, and accompanied by appropriate statistical treatment. The information provided by Amylogene indicates that the samples analysed for composition purposes were derived from two growing seasons, namely 1996 and 1997. In each year, three trials sites were used.

544. The SCP guidance document also indicates that that the specific analyses should be appropriate to the crop. In the case of potatoes, five constituents have been identified as important for compositional comparison, namely dry matter, sugars (especially reducing sugars), protein, vitamin C and glycoalkaloids (OECD, 2002). However, several other key constituents were also identified including starch, fat, fibre, minerals, protease inhibitors and lectins. Amylogene submitted information on the five constituents recommended by the OECD Taskforce on Novel Foods and Feeds as well as the majority of the other constituents, the notable exceptions being protease inhibitors and lectins.

545. The results from the compositional analyses indicate that the majority of the constituents are produced in similar levels in the GM and non GM lines. The levels of three constituents, nitrate, glycoalkaloids and fibre, appear to differ between the two potato varieties in one of the growing seasons only and thus the differences do not appear to be specific to the variety of potato. The analyses also identified several constituents that show statistically significant differences between the GM and non GM potatoes, including dry matter (may be related to changes in yield), vitamin C and sugars. These differences are consistently observed over the two growing seasons examined and thus appear to be variety specific.

546. To determine if the differences observed in the levels of vitamin C and sugars are likely to be biologically significant, the levels observed in both potato varieties were compared to the levels of the constituents present in the non GM potatoes grown under different environmental conditions. This analysis appears to have been presented for the information from the 1996 trial only. In all cases the levels of the three sugars tested and vitamin C fall within ranges present in other varieties of potato that are readily available. It is therefore unlikely that the detected differences in composition pose a threat to human health and safety. Further information from another growing season (ie 1998) is therefore not necessary to ensure the validity of the safety assessment.

547. Amylogene undertook a co-variant analysis on part of the data set, apparently to analyse possible effects of the reduced yield on levels of particular constituents. Given this approach, it is reasonable to analyse the data from the two growing seasons both as single data sets as well as a combined data set. However, this analysis is not necessary to ensure the validity of the safety assessment for the reasons outlined above.

548. Other Issues

February 1999 EC-67/Att 84 4: the SCP requested information about the fractionated potato products:

**Concerning the overall use of the GM plant it would be beneficial to have a fractionation scheme showing how by-products arise and the conditions used for extraction. Relevant analysis of each by-product entering the food chain is required.**

549. General information on the fractionation of the potato was provided in the original application. The genetically modified potato will be used primarily for starch production. By-products of starch production are pulp (solid remains of the potato after the starch has been extracted) potato juice (liquid from the potatoes) and potato water (water used to wash the potatoes). The juice and water are used for irrigation and the pulp is used for cattle feed. The potato and its products are not intended to be used for human food, nor is it intended that the whole tubers be used for animal feed.

550. The safety assessment, addressing the molecular characterisation, properties of any newly introduced substances and compositional analysis, has not identified any health and safety issues arising from the genetic modification. The only compositional difference of significance is the type of the starch, with the genetically modified potato having high levels of amylopectin. However, a range of conventional crops produce the amylopectin type of starch (eg maize) and amylopectin type starch is also chemically isolated from potatoes. Therefore, the safety of this type of starch is well established (see also response to questions on Toxicity and animal feeding trials).

551. The information requested on fractionation of by-products and analysis of the by-products entering the food chain was not necessary to support a valid safety assessment.

552. **Overall Conclusion to Question 25:** The SCP was not initially provided with adequate information to enable a valid safety assessment to be conducted. However, much of the information requested by the SCP would not have had an impact on the conclusions of the safety assessment. It also should be noted that in seeking information from Amylogene, the SCP could have anticipated much of the data required and requested this from the company in a more timely and coordinated manner.

553. In conclusion, the totality of the information provided to the SCP up to 20 July 2000 was sufficient to enable a valid safety assessment to be conducted. The information requested after this date (and much of the information requested prior to this date) is unlikely to influence the conclusions of the safety assessment.

**Dr. Snape**

554. The comments below relate to Question 25 concerning only the molecular characterisation of exhibit EC-067. These were examined relative to the EFSA-Q-2003-005 guidance document produced by the Scientific Panel on Genetically Modified Organisms.

555. **Exhibit EC-067:** Potato event EH92-527-1 was produced, using plasmid pHoxwG, by means of Agrobacterium-mediated gene transfer. The T-DNA of the plasmid pHoxwG used for transformation carries two expression units: one contains the potato gbss gene in antisense orientation and one contains the nptII antibiotic resistance gene. Potato event EH92-527-1 contains one functional transgenic locus containing one copy of the gbss antisense gene. Information relative to the molecular characterisation of EH92-527-1 has been found primarily in EC-067 attachments 14, 18,

20, 21, 36, 37, 39, 41, 42, 43, 44, 60, 82, 107, 108, 111 and 113. A series of comments and questions are numbered below. This is followed by a conclusion proposing answers to Question 25:

556. EC067 – attachment 021: (1) The southern blot analysis seems to suggest the presence of three copies of the gbss promoter (figure 3) and of the gbss gene (figure 8) in wild-type Prevalent potatoes. This contrasts with the results obtained in figure 6 suggesting that only one copy of the gbss gene is present in wild-type Prevalent (one hybridization signal/band in lane D).

557. EC067 – attachment 021: (2) No molecular information is provided on the nptII gene integrated into EH92-527-1. This reviewer would suggest to re-probe membranes shown in figure 3,6 and 8 with an nptII probe.

558. EC067 – attachment 036: (3) PCR analysis of the T-DNA integrated into EH92-527-1 suggests the loss of parts of the left T-DNA sequence in EH92-527-1. More information would be required to characterise the extent of the T-DNA deletion at the left border.

559. EC067 – attachment 037: Shows the absence of transgenic sequence in the chloroplastic genome of EH92-527-1 using Southern blot analysis and "labelled fragments" of the pHoxwG vector used as probes.

560. EC067 – attachment 039: (4) Shows the absence of vector backbone sequences in EH92-527-1 using a "probe cocktail" from a ~8000 nt BglII fragment described in attachment 60. The use of smaller, well defined probes from the backbone (nptIII gene etc...) would have provided more accurate information.

561. EC067 – attachment 041 to 44: Provides maps and the entire sequence of the pHoxwG vector used for potato transformation.

562. EC067 – attachment 060: It is mentioned that new experiments will be undertaken to detect backbone sequences and to sequence genomic flanking regions. (5) This reviewer disagree with the following sentence on page 8 of the PDF file "Annex 21 does not give a clear cut case when it comes to unwanted vector sequences in other parts of the genome of EH92-527-1 than associated with the inserted integrated DNA". The absence of hybridisation signal in the Southern blot hybridisation conducted in Annexe 21 (i.e. attachment 39) is enough to prove that no backbone sequence is integrated anywhere in the genome of EH92-527-1 (with the limitations associated with the efficacy of using a probe cocktail).

563. EC067 – attachment 86 and 113: (6) Suggests that the sequencing of genomic flanking regions has been performed. This reviewer was unable to find this information among the attachments presented.

564. EC067 – attachment 111: (7) This reviewer could not find the molecular analysis showing that EH92-527-1 exhibited "genotypic stability" as claimed page 7. Comparative Southern blot analysis of primary transformants and plants produced through vegetative multiplication is required to assess the structural stability of a transgenic locus.

565. **Conclusion – response to Question 25:** Since the first submission in 1996, the following molecular information about EH92-527-1 has been established:

- EH92-527-1 contains one functional locus containing a single copy of the gbss gene in antisense orientation.

- Some deletion has occurred in the left T-DNA border region.
- Transgenic sequences outside the T-DNA are unlikely to have been integrated into EH92-527-1.
- Transgenic sequences from pHoxwG are unlikely to have been integrated into the chloroplastic genome.

566. I would suggest that more information is still required about:

- The sequence of the transgenic locus especially around the deleted left T-DNA region. The proposed architecture of the transgenic locus currently relies only on PCR-based studies.
- The sequence of the flanking plant genomic regions.
- Additional Southern analysis (using small well defined probes) to confirm the absence of backbone sequences into the genome.
- The molecular analysis of plants propagated vegetatively to demonstrate the structural stability of the transgenic locus across multiplication cycles. The stability of the altered starch composition trait can be used to estimate the genotypic stability of the gbss expression unit as only one transgene copy is present in EH92-527-1.

567. Additional information may have been submitted to the EC by the company. Experts should examine if this information answers some of the requests formulated above.

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

**Question 26: Given the information before the Panel, including the notification (EC-70/At.1-3), was the information regarding molecular characterization of this product requested by the Dutch CA and by the UK (EC-70/At.4, 7, and 8) necessary to ensure that conclusions of the safety assessment were valid?**

**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?**

**Dr. Nutti**

568. Based on the information provided by the applicant in EC-070/At.8 and 13, and the analysis done by the EC SCP and 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, the notifier carried out animal feeding studies with rainbow trout, quail, broilers and lamb. In all these studies, no differences were detected among the animals fed conventional and GM oilseed rape based feeds. Therefore, it is concluded that the studies requested by the Netherlands were not necessary to ensure the product's safety assessment.

**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?**

**Dr. Nutti**

569. Based on the information provided by the applicant in EC-070/At.1 to 3, and the analysis done by the EC GMO Panel, where it was stated that GT73 oilseed rape is as safe as conventional oilseed rape for humans and animals, and in the context of its proposed use, I concluded that no additional experimental studies were necessary, I understand that the additional information provided by the notifier was not necessary to ensure that conclusions of the safety assessment were valid.

**Dr. Andow**

Necessity of additional information on monitoring plan:

570. In this answer I will address only the monitoring issue. Here the question becomes "is a monitoring plan necessary to ensure that conclusions of the safety assessment were valid?" The scope of the notification is for import and use of GT73, excluding the cultivation of varieties derived from GT73.

571. The monitoring issues are summarized by the notifier in EC-70/At.85.

6. Monitoring

*AUSTRIA: The scenarios set out at point 2.3 and the properties of rapeseed oil also facilitate the appearance of the product in various conventional rapeseed oil cultivation areas or adjacent locations, even though probably only as a small proportion. Nevertheless, this fact must be accordingly taken into account by consideration in a monitoring programme in accordance with appendix VII f the guideline 2001/18/EG. However, this is not allowed for on the part of the applicant (cf. test report by the competent Dutch authorities).*

*DENMARK: The monitoring plan should include observation on dispersal and gene transfer to oilseed rape and wild relatives.*

*ITALY: The company shall not only indicate the main places of entrances in each country but also the storage location and the way of the transport; this is due to the need of guarantee an efficient monitoring plan. In any case the processing plants should be nearby the places of entrance and every measure to avoid the dispersal of grains into the environment must be ensured.*

*SPAIN: The current scope of this application for consent to place GT73 oilseed rape on the market includes both import and processing in the European Union, excluding cultivation. Nevertheless, assuming that an unintentional release could be raised, the Spanish Competent Authority considers that a post-market monitoring plan is needed to consider this aspect. The monitoring plan should include sampling in order to carry out studies on transgene expression on wild relatives, conventional oil seed rape crops, volunteers growing across the transit way during import and processing.*

UK: *The UK requests to monitor for the presence of feral GT73 oilseed rape at the points of import and processing, and between those locations and proposes to establish an action plan should feral GT73 be identified ... The proposal for post-market monitoring is not adequate. As outlined above, the environmental risk assessment assumes that seed will not be spilled, so the post market monitoring plan should test this assumption. The UK requests that the applicant gives further consideration to the post-market monitoring plan, which should include (i) monitoring for the presence of feral GT73 oilseed rape at the points of import and processing, and between those locations; and, (ii) proposals for action should feral GT73 be identified.*<sup>93</sup>

572. The full context for understanding Austria's concerns is provided in EC-70/At.77, 25 March 2003, Objections raised by Austria, and is reproduced below. Austria is concerned about accidental release during transport or processing and subsequent invasion of other agricultural systems.

- 2.3 Ecology: Importing the product for processing and use for food and animal feed is no guarantee that GT73 will not be released into the environment. Experiences and checks in Austria during the last few growing seasons have shown that genetically modified seeds can come into circulation as a result of accidental or technically unavoidable contamination (e.g. in the transport or processing stage). In the case of oilseed rape, with its specific characteristics (ability to survive, potential for dispersal and outcrossing), there is a possibility of it spreading into agricultural ecosystems.

573. This could therefore affect the possibilities for coexistence between different agricultural production systems with and without GMOs. The product cannot be approved until there is EU-wide clarification of the possibilities for coexistence and a legally binding framework (e.g. specifying threshold values) has been defined.

574. The full context for understanding Italy's concerns is provided in EC-70/At.71, 24 March 2003, Objections raised by Italy. Italy requests monitoring to provide a means for managing risks associated with AMPA.

2) As for the previous notification C/ES/00/01, there is an extensive discussion of possible effects on animal and human health (due to antinutritional factors), but there is no data on the possible content of glyphosate and/or metabolites (AMPA). In this regard, we note that event GT73 contains a gene specific for the degradation of glyphosate into AMPA, but see no discussion of the possible consequences.<sup>94</sup>

575. The Spanish CA expands on their concerns in EC-70/At.75, 25 March 2003, Request for additional data from Spain. Spain is concerned about accidental releases during transport or processing.

Specific actions to avoid unintended dispersal

The Company should strengthen the operating procedures to ensure that seeds are not spilled during transport and processing. These requirements should be included in the consent of the product.

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<sup>93</sup> Page 25. This and all other page references to EC documents in the answer refer to the page of the pdf file.

<sup>94</sup> Page 1.

576. The full context of the concerns of the UK are given in EC-70/At.74, 25 March 2003, Request for additional data from UK. The UK is concerned about accidental release and suggests that the monitoring plan test the assumption that there will be no spillage, either during importation, transport or processing.

2. Procedures to avoid seed spill. A central argument used in the environmental risk assessment of this GMO is the assumption that the rape seeds will not be released into the environment. However, if there is spillage of seed between import and processing this may lead to the presence of transient populations of feral GT73 oilseed rape. **The UK requests further details from the applicant concerning the standard operating procedures that will be used to ensure that seed is not spilled during transit.** Acceptable procedures to minimize seed spill should be a condition of any consent issued – this could restrict import and processing to specific locations and require monitoring for seed spill between the sites of import and processing (see below).<sup>95</sup>

5. Post-market monitoring plan. The proposal for post-market monitoring is not adequate. As outlined above, the environmental risk assessment assumes that seed will not be spilled, so the post-market monitoring plan should test this assumption. **The UK requests that the applicant gives further consideration to the post-market monitoring plan, which should include (i) monitoring for the presence of feral GT73 oilseed rape at the points of import and processing, and between those locations; and, (ii) proposals for action should feral GT73 be identified.** The proposal for post-market monitoring also includes a request to end users of GT73 grain to inform the regulatory authorities of any adverse effects that are attributable to the use of the GMO. The UK considers that the applicant should be more proactive in obtaining this information and should be combined with a reporting requirement. **The UK requests that the consent holder approach end users directly to ask if they have observed any effects to cover general surveillance and the results of this should be reported to the competent authorities at intervals of 6 months for the first 3 years of the consent.** The report should list the requests that have been made to end users, the proportion that elicit a response and what, if any, unexpected effects were observed (adverse or otherwise).<sup>96</sup>

577. These concerns of the UK were initially articulated by the scientific Advisory Committee on Releases to the Environment (ACRE) in EC-70/At.69, 10 March 2003, ACRE Advice to the Secretary of State for Environment, Food and Rural Affairs, Scottish Ministers, Ministers of the Welsh Assembly Government and the Department of Environment (Northern Ireland). Their advice and reasoning is repeated here.

Primary Advice:

- 3. The post market monitoring plan should include monitoring for spillage of GT73 seed during import, transportation and processing and test for the establishment of feral populations of GT73 oilseed rape. The plan should also include appropriate emergency plans should such populations be identified.

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<sup>95</sup> Page 2.

<sup>96</sup> Page 2.

Post market monitoring plan:

- The post market monitoring plan is a new requirement under Directive 2001/18/EC. The plan provided by the notifier is brief and limited. On the basis of the risk assessment for GT73 Monsanto do not propose to carry out any case-specific monitoring. ACRE agree with this conclusion as the risk assessment does not identify any risks in relation to human health or the environment from the use of GT73 oilseed rape as defined in the notification (i.e. for import and processing).
- Directive 2001/18/EC places a requirement to provide a monitoring plan for the occurrence of adverse effects not anticipated in the environmental risk assessment. ACRE consider that the proposal in response to this requirement put forward by the notifier is unsatisfactory. In particular the plan does not take into account monitoring of the occurrence and effects of seed spill during transportation and processing of the oilseed rape seed. Based on current experience regarding movement of non-GM oilseed rape in the UK seed spill is likely to occur and will result in the survival and establishment of feral oilseed rape populations and their hybridisation with crop and other feral populations. It is accepted that the risk of harm to human health and the environment from seed spill is low, however there is an issue, in the UK, regarding the segregation of transgenic and non-transgenic material. Hence, ACRE request that Monsanto review their monitoring plan taking into account methods of monitoring and reporting seed spill, incorporating a proposal as to how seed spill may be controlled. It should also include plans for monitoring and controlling establishment of feral populations as a result of seed spill.

578. In addition, Sweden raised a monitoring issues in EC-70/At.67, 28 February 2003, Swedish Board of Agriculture, which is repeated below. Sweden has focused on procedures to report adverse effects.

A. Monitoring plan

1. The monitoring focuses on the identification, checking and traceability of GT73, whereby Monsanto Europe is to:
  - pass information to the traders and users within the processing industries in order for them to be able to identify any negative effects on the environment or on human health and request these users to inform the Competent Authorities about any identified negative effects on the environment or human health which are assumed to have come about from the use of GT73,
  - to inform the European food industries directly when the product has been approved and to request operators in food chains to inform the Competent Authorities about any identified negative effects on the health of animals, which are reported to them by farmers or other users, and which are assumed to have come about from the use of GT73,
  - immediately inform the Commission and Competent Authorities during the consent period if the company receives information about negative effects of the product.

579. Moreover, although Norway is not a part of the EC, Norway submitted comments related to monitoring in EC70/At.78, 25 March 2003, Comments from Norway. Norway merely notes that there should be a monitoring plan.

Although required according to Directive 2001/18/EC, there is no monitoring plan attached to the application. The Competent Authority of the Netherlands recommends an approval on the condition that the notifier gives a yearly report of the sold quantities of line GT73 in each country. We will comment on the need for additional monitoring when we have finished our assessment of the risks for human health and the environment.

580. Notwithstanding that a monitoring plan is required by Directive 2001/18/EC, it is still possible to consider if a monitoring plan is necessary from a scientific standpoint, and if so, what kind of monitoring plan could appropriately address the potential risks.

581. Before doing so, I will establish that in the SNIF to COM on 16 January 2003, the notifier proposed a plan that must be considered a case-specific monitoring plan. This is a critical step, because had the notifier not proposed such a monitoring plan, then the issue would hinge on whether any monitoring plan is needed. Instead, the issue will hinge on the adequacy of the proposed monitoring plan. This is a substantially more complex argument.

582. In EC-70/At.54 (16 January 2003 SNIF), the notifier states that unintended release can occur during import, transport and processing. The notifier suggests that because most grain is crushed near seaports and modern equipment is used that there is little chance of unintended release.

7. Measures suggested by the notifier to take in case of unintended release or misuse as well as measures for disposal and treatment

This application is for consent for import and use in the EU of GT73 as any other OSR, but do not include the cultivation of varieties in the E.U. Therefore an unintended release would be more likely to occur during import, processing and transportation of GT73 grain. However, most OSR grain imported into the E.U. via European sea ports is immediately crushed in nearby crushing facilities and modern methods of transportation and grain handling minimize losses of grain. Therefore, there is little chance of germination of grain resulting in the development of mature plants of GT73 in the E.U. environment.

GT73 is intended for use by oilseed producers, in the same manner as other oilseed rape varieties. The measures for waste disposal and treatment for GT73 products are the same as those for other oilseed rape products.<sup>97</sup>

583. In EC-70/At.54 (16 January 2003 SNIF), the notifier argues that there is no need for post-release monitoring. First, two years of monitoring volunteers in Canada and the US indicated that there were no greater numbers of volunteers than non-transgenic rape and that the transgenic volunteers could be controlled by other herbicides. Second no adverse effects of GT73 have been identified.

584. Previous commercializations of GT73 have been alongside a stewardship program, which provides a communication channel if an unanticipated adverse effect would occur.

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<sup>97</sup> Page 5.

(f) Aim of post-releases monitoring

In the case of the field testing performed in Canada and the US, the fields were monitored for volunteers during a period of two years and volunteers were chemically removed.

Since no adverse effects of GT73 have been identified (see section 29), no requirement for case-specific post-release monitoring is indicated, which is consistent with approvals granted in other world areas.

GT73 has been commercialized alongside stewardship programmes involving downstream stakeholders in the use of this oilseed rape, in order to ensure the implementation of good agricultural practice in its cultivation and a channel of communication in the unlikely event that unanticipated adverse effects might occur.

(g) Duration of post-releases monitoring

In the case of the field testing performed in Canada and the US, the fields were monitored for volunteers during a period of two years.

(h) Conclusions of post-release monitoring

The monitoring conducted after the Canadian and US field releases showed no differences in volunteer numbers between GT73 and conventional OSR varieties in the number of volunteers, and confirmed that GT73 volunteers can be effectively controlled. No unanticipated effects have been observed since the commercialization of GT73 in other world areas, nor during the many years of field-testing inside and outside the E.U.<sup>98</sup>

585. In EC-70/At.54 (16 January 2003 SNIF), the notifier indicates that the monitoring plan will focus on general surveillance for unanticipated adverse effects.

D. INFORMATION RELATING TO THE MONITORING PLAN-  
IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED  
TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT  
SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION  
MONITORING PLAN

2. Identification of the occurrence of adverse effects of the GMO or its use on  
human health or the environment which were not anticipated in the E.R.A.

The environmental and human health safety assessment for GT73 did not identify any specific risks related to its placing on the market during storage, processing and other uses. Therefore the monitoring plan for GT73 is focused on general surveillance for unanticipated, adverse effects.<sup>99</sup>

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<sup>98</sup> Page 19.

<sup>99</sup> Page 20.

586. In EC-70/At.55 (16 January 2003 SNIF), the notifier indicates that there will be no special measures taken to prevent escape or misuse of the product nor will there be any specific instructions or recommendation for storage or handling.

#### Part II. Additional Relevant Information

5. The measures to be taken in the event of the escape of the organisms in the product or misuse of the product.

GTOSR is targeted for use by oilseed producers, in the same manner as other OSR varieties. Dissemination of OSR plants into non-agricultural environments, such as roadsides and other semi-managed areas, occurs currently. The information presented in this application establishes that the frequency of dissemination of GTOSR will be similar to other OSR, and no special measures will be taken to prevent dissemination.

6. Specific instructions or recommendations for storage and handling of the product.

Since GTOSR varieties have been demonstrated to be substantially equivalent to other OSR varieties, apart from their tolerance to glyphosate, no specific instructions or recommendations for storage and handling are envisaged.<sup>100</sup>

587. Lastly, the notifier submitted with the 13 December 2002 notification to the Netherlands CA an Annex VII, Monitoring Plan (EC-70/At.51) in which they propose that no case-specific monitoring is needed.

588. However, in the same appendix they propose three measures to identify potential unanticipated adverse effects. All three measures are specific to GT73, so these measures must be considered a proposal for case-specific monitoring, even if the notifier and ACRE (paragraph 577) do not recognize them as such. The specifics of this proposal follow. It should be clear that although this proposal parallels the concerns of Sweden (paragraph 578), it is substantially different.

2. Identification of the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the e.r.a.

Since the notification of GT73 oilseed rape is for consent for import only, and since the majority of use of this oilseed rape will be for human and animal consumption (see Annex IV, A.4.), it follows that unanticipated affects are most likely to be manifested as a result of this use. Therefore, it is proposed:

- To provide traders and processors of bulk mixtures of oilseed rape grain, likely to contain GT73 oilseed rape grain, with product information about GT73 oilseed rape grain. Traders and processors will be requested to inform the relevant authorities of any adverse effects on the environment or human health, which they consider to be attributable to GT73 oilseed rape grain.
- To inform the European feed industry directly, by way of a public announcement, of the consent for placing on the market of GT73 oilseed rape at such time as it appears

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<sup>100</sup> Page 66.

in the Official Journal of the rapporteur Member State. Monsanto will also offer to meet with interested operators to discuss the safety and general characteristics of the product. Operators in the feed chain will be requested to inform the relevant authorities of any adverse effects on animal health reported to them through farmers or national feed associations, which they consider to be attributable to the feed use of GT73 oilseed rape grain.

- For the duration of the authorization of GT73 oilseed rape, to immediately inform the Commission and the Competent Authorities for Directive 2001/18/EC, of any reports of adverse effects, which come to the attention of Monsanto, so that any reports can be further investigated by the appropriate authorities.

589. Although it may be true that the notifier made a similar monitoring proposal in the 16 January 2003 SNIF, I could not find this clearly stated in the SNIF. However, under the assumption that any item in the notification to a country CA is automatically a part of the submission to the COM, it must be concluded that **there was a case-specific monitoring plan proposed in the 16 January 2003 SNIF.**

590. Are the additional requests made by the member nations regarding the monitoring plan necessary to ensure that conclusions of the safety assessment were valid? To argue this question, I will first consider if concerns over inadvertent escape are valid, then consider if there are valid reasons for distinguishing GT73 from other rape varieties, then discuss the adequacy of the proposed case-specific monitoring plan, and finally I will address the specific monitoring advice provided by the various member countries. In addition, I will consider if there was any change in the proposed monitoring plan, based on an examination of the new information submitted by the notifier on 5 August 2003 (EC-70/At.92).

591. Concerns that rape seed can escape during import, transport or processing are valid. This was first brought to scientific attention for rape seed in 1995 in the UK.<sup>101</sup> Moreover, using standard modern equipment, it is simply not possible to ensure that spillage will not occur. The concerns of Denmark (paragraph 571), Austria (paragraph 572), Spain (paragraph 575) and the UK (paragraph 576) begin with this concern.

592. Should transgenic GT73 be distinguished from conventional rape varieties? Here the various countries express different perspectives. Denmark (paragraph 571), Spain (paragraph 575), the UK (paragraph 576) and Norway (paragraph 579) do not link their request for additional monitoring to a specific risk. Austria is concerned about potential contamination of other agricultural systems with undesired transgenes (paragraph 572), Italy is concerned about potential effects of glyphosate metabolites (paragraph 574), and Sweden is concerned that a monitoring system is set up so that adverse effects, should they occur, can be identified (paragraph 578). In addition, the UK (paragraphs 576 and 577) suggests that monitoring should test the assumption of the notifier that seed will not be spilled.

593. Without a connection to a specific risk or the risk assessment, requests for monitoring GT73 cannot be distinguished from conventional rape varieties, and the requests and concerns of Denmark, Spain and Norway cannot be scientifically justified on the basis of the written record received. Austria's specific concern and the reasoning behind the UK requests are scientifically justified, and both would require that GT73 is distinguished from conventional rape varieties. I was unable to

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<sup>101</sup> Crawley, M.J. & Brown, S.L. (1995). Seed limitation and the dynamics of oilseed rape on the M25 motorway. Proc. R. Soc. Ser. B.-Bio, 259, 49-54.

determine if Italy's concern had been treated in the notification (13 December 2002) or the SNIF (16 January 2003). If it has not been adequately assessed, then Italy's concern is scientifically justified, and would distinguish GT73 both from conventional rape varieties and from glyphosate itself (because the plant metabolite AMPA would be less of a concern on non-GMHT oilseed rape crops). Sweden's concern is that the monitoring plan be sufficient to identify unanticipated adverse effects.

594. Adequacy of proposed case-specific monitoring plan (EC-70/At.51, Annex VII, Monitoring Plan). The test for adequacy is whether the proposed plan would meet the aims proposed by Sweden, Austria, the UK and Italy. The first measure (paragraph 587)<sup>102</sup> partially meets Sweden's concern, but does not address the concerns of Austria, the UK or Italy. The second measure (paragraph 587)<sup>103</sup> meets none of these countries concerns. The third measure (paragraph 587)<sup>104</sup> partially meets Sweden's concern, but does not address the concerns of Austria, the UK or Italy. Thus the proposed monitoring plan does not meet the concerns of the Sweden, Austria, the UK or Italy.

595. Specific monitoring advice from the member countries is incomplete and too inflexible. By incomplete, I refer to the criteria for monitoring in my response to question 24a. By inflexible I refer to the recommendations that the notifier monitor rapeseed cultivation areas, gene dispersal, gene transfer, studies on transgene expression in wild relatives and conventional oil seed rape crops, and sampling of volunteers. Meeting the concerns of Sweden, Austria, the UK and Italy do not require such an intensive monitoring plan. A general principle of GMO risk assessment is that risk is proportional to the scale of release.<sup>105</sup> Low frequency releases at small spatial scales will have lower risk, and the monitoring system should reflect this.

596. The revised monitoring plan (5 August 2003, EC-70/At.92, Appendix 9)<sup>106</sup> falls well short of the meeting the concerns expressed by Sweden, Austria, the UK and Italy, and meets even fewer of the criteria for an adequate monitoring plan that the original proposal (13 December 2002, EC-70/At.51).

597. In EC-70/At.85, the notifier makes an effective argument<sup>107</sup> that grain entering the main gateways and crushed in the port areas is very unlikely to establish in the environment. Hence it could be suggested that the concerns of Austria, the UK and Italy could best be met by concentrating monitoring activities in places other than these main gateways and crushing facilities, focusing on those for which escape may be more likely (worst cases). By monitoring a few potential worst cases,

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<sup>102</sup> To provide traders and processors of bulk mixtures of oilseed rape grain, likely to contain GT73 oilseed rape grain, with product information about GT73 oilseed rape grain. Traders and processors will be requested to inform the relevant authorities of any adverse effects on the environment or human health, which they consider to be attributable to GT73 oilseed rape grain.

<sup>103</sup> To inform the European feed industry directly, by way of a public announcement, of the consent for placing on the market of GT73 oilseed rape at such time as it appears in the Official Journal of the rapporteur Member State. Monsanto will also offer to meet with interested operators to discuss the safety and general characteristics of the product. Operators in the feed chain will be requested to inform the relevant authorities of any adverse effects on animal health reported to them through farmers or national feed associations, which they consider to be attributable to the feed use of GT73 oilseed rape grain.

<sup>104</sup> For the duration of the authorization of GT73 oilseed rape, to immediately inform the Commission and the Competent Authorities for Directive 2001/18/EC, of any reports of adverse effects, which come to the attention of Monsanto, so that any reports can be further investigated by the appropriate authorities.

<sup>105</sup> NRC (National Research Council). (1987). *Introduction of recombinant DNA-engineered organisms into the environment: key issues*. National Academy Press, Washington, DC.

<sup>106</sup> Pages 17-26.

<sup>107</sup> Page 26.

it can be argued that all other cases are likely to have even lower risk. The suggestions by Sweden (paragraph 578) have not been addressed yet.

598. Monitoring is a form of risk management, and might be argued to be independent of the risk assessment process. However, in this specific case, issues associated with monitoring are intricately tied to the issues associated with risk assessment, in that the risks associated with unanticipated possible hazards can be reduced to acceptable levels with the appropriate monitoring plan, as suggested by Sweden. For GT73, specific concerns include contamination of other agricultural systems and possible effects of glyphosate metabolites. Moreover, monitoring can be used to confirm critical assumptions in the risk assessment (Annex VII, Directive 2001/18/EC), which is suggested by the UK for this case. Hence, from these perspectives, it can be concluded that **a monitoring plan is necessary to ensure that conclusions of the safety assessment were valid.** However, the monitoring plans advised by the countries are on balance overly specific and do not allow the notifier sufficient latitude to suggest alternative approaches that would address the concerns in ways that may be more cost-effective.

599. The Member States requested information related to the monitoring plan on 28 February 2003 (Sweden) and 24/25 March 2003 (Austria, Italy, Spain, United Kingdom). I have assumed that the monitoring plan was submitted to COM with the SNIF on 16 January 2003. The earliest date of submission of this plan was 13 December 2002. Using the later date, it took the Member State 43-68 days to respond with comments. Using the earlier date, this would be 77-102 days to respond (including the winter holidays). These do not seem to be unreasonable lengths of time for a response.

**Bayer Liberty Link oilseed rape (T45 & Topas 19/2)**  
**C/GB/99/M5/2 (EC chronology 72)**

**Question 29: Given the information before the Panel, including the notification (EC-72/At.1-3) and additional information provided by AgrEvo (EC-72/At.7-9), were requests for more information (EC-72/At.5, 6, and 11-13) concerning feeding studies, chemical compositional data and potential interactions of transformation events in the stacked product necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

600. Based on the information provided by the applicant in EC-72/1-3 and additional information at EC-72/At.7-9 and the minutes of the Sixty-Second Meeting of ACRE, when it was verified that there were a number of inconsistencies in the molecular data provided and the dossier was rather impenetrable. Also, too much important material was buried in annexes rather than being in the core dossier and the descriptions of the plasmid maps and analyses did not correspond with the data as presented. Further concerns were expressed about deficiencies in the molecular studies carried out to characterize the inserted DNA, although it was accepted that the appropriate experimental data may have been supplied somewhere in the notification dossier but it was not immediately obvious where it might be. Therefore, I understand that ACRE and the UK SC requests for more information were necessary to ensure that conclusions of the safety assessment were valid. It is important to point out that ACRE was not being asked for formal advice at that stage, only guidance; therefore, no conclusions were drawn at the time.

*Stoneville BXN cotton (10215, 10222, 10224) (formerly held by Aventis and Calgene)*  
*C/ES/99/01 (EC chronology 73)*

**Question 30: Given the information before the Panel, including the notification (EC-73/At.1), was additional information regarding environmental impact tests, feed safety, residue analysis, monitoring for herbicide resistance requested by Spain (EC-73/At.3) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

601. This question could not be properly answered because the complete notification was not made available to me (At. 1 corresponds to the notification index only). However, the answers to the questions, which are presented in At.4, seem to be satisfactory as far as food safety is concerned (question 7). Nevertheless, the entire evaluation process is, by the chronology made available, at its initial stage, so conclusions on the validity of the safety assessment cannot be drawn.

**Dr. Andow**

Necessity to ensure validity of safety assessment

602. I will address items 8, 9 and 11 as listed in EC-73/At.03 (15 July 1999, proposed amendments to 3 May 1999 notification made by lead CA). These relate to environmental impact tests, herbicide studies, and resistance evolution. The issue is to determine if this additional information is necessary to ensure valid conclusions of the safety assessment.

8. The results concerning the environmental impact of these genetically modified plants refer almost exclusively to studies conducted in the USA, Japan, Argentina, etc. (page 37). The results of tests conducted in Spain and Greece should also be included, considering that in some cases the environmental conditions cannot be extrapolated.

9. Indicate where and when the survival study described on page 38 was carried out. These studies were conducted on two lines other than the lines covered by this application.

11. Provide toxicological and ecotoxicological studies on the herbicide used on this type of crop.

13. As regards points 11 and 12, the results of the studies must be indicated as a function of the different doses of product applied, as was the case in various phenological studies on cotton plants.

In addition to clarification of these points, the National Biosafety Committee considered that the company should propose specific methods for management, monitoring and handling of this crop in order to reduce the possibility that resistance to this herbicide could emerge.

603. Similar concerns are reiterated in EC-73/At.12, the 26 September 2003, analysis of the 16 January 2003 notification by the Spanish NCB.

604. The full text of the original notification is not available. EC-73/At.01 contains only the table of contents of the full notification submitted to the Spanish CA on 3 May 1999. From an examination of the appropriate parts of this table of contents (below), I conclude that no environmental impact studies were reported, no herbicide or residue toxicity or ecotoxicity tests were reported, and no proposal to manage, monitor and handle the crop to reduce the risk of herbicide resistance in weeds was made. There are no headings in the table of contents for some of these topics, and the number of pages that could be devoted to these topics is <2 pages.

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605. The full text of the SNIF (19 March 2003) and the updated notification (15 January 2003) are not available in the materials provided.

606. This requires me to assume that the issues noted in 30.01 were not addressed in any of the submissions by the notifier, and to answer the question without reference to the specifics of the case.

607. Environmental impacts. Data from the US, Japan, Argentina, etc on page 37 of the original notification must refer to cotton reproduction and seed and pollen dispersal rather than environmental impacts. The table of contents indicates that page 37 addresses these topics, not environmental impacts. Some data on environmental impacts is needed to support a scientific finding of safety. It must be concluded that the request for environmental impact tests is necessary to ensure that conclusions of the safety assessment are valid. How much testing is necessary, however, is not addressed by this conclusion.

608. In the hypothetical, we can suppose that environmental impact data from US, Japan, Argentina, etc. were available and presented as data, rather than summary conclusions. Would this be sufficient to meet the needs for completing a scientifically valid assessment of environmental impact? In other words, is it necessary to have local data from Greece and Spain to complete a scientifically valid assessment of environmental impact? In general, this would be difficult to answer in the abstract, because depending on the impact being assessed, it may be possible to generalize across continents. For example, a species that is invasive in one location, such as Monterey pine in South Africa or the seaweed caulerpa in the Mediterranean Sea, is predicted to be invasive in all other parts of the world with similar climate. For transgenic crops, however, this can be answered in general, at least up to and including the present time (January 2005). There is still not enough scientific information to know how environmental impact of transgenic crops generalizes across continents. Indeed, the commitment to case-specific risk assessment is made because it is not yet clear how to generalize across crop, transgene, or receiving environment when it concerns environmental risk. Hence, it must be concluded in the hypothetical that the request for environmental impact tests conducted in relevant environments in EC countries is necessary to ensure that conclusions of the safety assessment are valid. Again, the question of how much testing is necessary is not addressed by this conclusion.

609. Herbicide and residue toxicity and ecotoxicity. Many of these issues may have already been addressed during the process of registering the herbicide for use on the untransformed crop. An issue that may not have been addressed in during herbicide registration concerns plant-specific herbicide metabolites. Thus, it is possible to conclude that the request for herbicide residue analysis is necessary to ensure that conclusions of the safety assessment are valid. However, it is also possible that this information is already available under pesticide registration laws, in which case, it may not be necessary.

610. Weed resistance risk, management and monitoring. I addressed this issue at some length in my response to question 24. The same argument holds here. Common groundsel (*Senecio vulgaris*) in Oregon is resistant to bromoxinil.<sup>108</sup> This is a common weed in Europe. The risk of resistance is real. It must be concluded that the request for monitoring for herbicide resistance is necessary to ensure that conclusions of the safety assessment are valid. Once again, however, this does not address the question of how much information on herbicide resistance is necessary.

611. I conclude that **the additional information regarding environmental impact tests and monitoring for herbicide resistance requested by Spain (EC-73/At.3) is scientifically justified and necessary to ensure that conclusions of the safety assessment are valid.** The requested information on residue analysis may be valid, or it may not be necessary (paragraph 609). However,

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<sup>108</sup> This is the only case of resistance to bromoxynil reported to date, [www.weedscience.org](http://www.weedscience.org), last accessed 22 December 2004. <http://www.weedscience.org/Case/Case.asp?ResistID=322>.

these conclusions do not address how much additional information would be necessary. Given the information before the Panel this quantitative issue cannot be addressed.

612. The information requested by Spain was on 15 July 1999 to material submitted by the notifier on 3 May 1999. This was 73 days to respond. This does not seem unreasonably long.

**Question 31: Given the information before the Panel, including the notification and the notifier's responses to questions (EC-73/At.2), was additional information regarding feed safety and compositional analysis (particularly related to nitrilase levels) (EC-73/At.3) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

613. Once again, this question could not be properly answered either, since the complete notification was not made available to me (At.1 corresponds to the notification index only). However, the answer to question 7 (nitrilase level in cottonseed oil) seems to be satisfactory. Also in this case, the entire evaluation process is, by the chronology made available, at its initial stage, so conclusions on the validity of the safety assessment cannot be drawn.

**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?**

**Dr. Nutti**

614. Based on the information provided by the applicant (EC-074/At. 01-15) and the request by the lead CA (EC-074/At.17), I can conclude that a lot of information was provided. However, I do not feel capable of judging if such material was enough to ensure that conclusions of the safety assessment were valid.

**Question 33: Given that Pioneer had notified this product under the regulation concerning the deliberate release of this plant (rather than the novel food regulation), were concerns raised by the lead CA about allergenicity of the truncated synthetic Cry1F protein relevant to the CA's assessment of this product (EC-74/At.17 and 33)?**

**Dr. Nutti**

615. Based on the information provided by the applicant (EC-074/At. 01-15) and the specific request on allergenicity by the lead CA (EC-074/At.17) and checking EC-074/At.05, page 54, my understanding is that the applicant provided information on the history of safe use of the donor organism and a general explanation on the other items, such as molecular weight of the protein, protein degradation in gastric fluids, expression levels of the protein and glycosylation of the protein. I believe that the applicant was supposed to present experimental data to support this general information.

616. Based on the further information presented by the applicant (EC-074/At.19) I can assume that the work carried out by Evans (1998) provides fundamental information on protein equivalence

(maize expressed core CRY1F and microbially-derived trypsinolyzed core CRY1F). However, I could not recover such information, presented on tables 10A, 11A, 12A and 13A in EC-074/At. 19) (I could not open the files on my computer). So, I cannot assess if the request from the lead CA was fulfilled.

617. From EC-074/At.33, I can infer that the second package of information provided by the applicant seems not to be sufficient as far as experimental data are concerned.

618. Examining EC-074/At.36, once again no complete annexes could be found (only pages identifying each annex can be visualized, but not the complete papers which were supposed to be there).

619. From all the above exposed, I do not have enough information to conclude that the concerns raised by the lead CA about allergenicity of the truncated synthetic Cry1F protein are relevant to the product's assessment. I would like to point out that this specific process called my attention since it is completely different from others I have reviewed, i.e., in my opinion, the requested information is not provided in a straight way, and experimental data are not presented in a clear way.

620. Based on EC-074/At.060, the comments from RIKILT on the work by T. Meyer (1999) concerning the need of correct updated data, full description of the methodology, and significant homology (according to FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2001) are, in my opinion, correct.

**Question 34: Given the information before the Panel, including the original notification and additional information provided by Pioneer (EC-74/At. 18-32, 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?**

**Dr. Nutti**

621. Based on EC-074/At.052\_SCI, Annex 1B, the request from the lead CA [xxx] was necessary.

622. Based on EC-074/At.052\_SCI, Annex 2B, the comments from RIKILT [xxx] are correct.

623. Based on EC-074/At.052\_SCI, Annex 6B, the comments from RIKILT [xxx] are correct.

624. Although I could not find the quail feeding study within the files, I would like to point out 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.

625. Examining EC074/At.058\_SCI, where the detection method is presented, so far as my knowledge goes, it seems to be correct (in my opinion, the notifier has to submit the method, the primers sequences and the reference materials, which was done). Although in EC074/At.060 the lead CA and RIKILT keep the same requests and comments, I do not feel capable of judging the raised issue of intellectual property and confidentiality.

**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?**

**Dr. Nutti**

626. It is not clear to me whether the mentioned EC field trials have already been done or are being requested. If they have already been carried out, the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003, Para 45) states that the location of the trials sites 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. Therefore, in this case, I understand that the field tests in EC and Chile can be considered as supplementary information to the previous tests carried out by the notifiers, although they were not necessary, (specially when we remember that the maize in question is for importation and processing).

**Dr. Andow**

627. Clarification from the Panel: Information provided by the notifier makes reference to field trials conducted in Chile that (it argues) provide compositional data on maize kernels that would be relevant to the evaluation of [?] products exported to the EC. See the notifier's response to questions from the CA, EC74/At.52\_SCI, page 6.

**Relevance to EC maize imports:**

628. The question with the response from the notifier is in EC-74/At.52\_SCI, 7 December 2001 (question originally posed 19 March 2001, EC-74/At.33), as follows.

Question 12

The applicant is requested to supply compositional data for kernels harvested from geographical areas representative for the commercial cultivation of maize 1507 (prior to export to the EU). Data for such areas should include multiple locations within an area and cover at least two seasons. In addition, these data should include the following treatments: transgenic 1507 maize plants treated with glyphosate, untreated transgenic 1507 maize plants, and untreated non-transgenic control plants.

Pioneer's reply to question 12

The locations for the field experiments that are described in the dossier, namely France, Italy, and Chile, would be representative for the cultivation areas exporting maize to the EU. GM maize plants cultivated at the EU test sites had been either treated or not treated with the herbicide glufosinate.

*Comment RIKILT*

RIKILT has not been convinced by Pioneer's statement that the EU locations would be representative of non-EU locations exporting maize and maize products to the EU.

629. It is clear that France and Italy cannot be locations representative of areas exporting maize to the EU, as they are part of the EU.

630. World maize export and import statistics are available from the US Department of Agriculture Economic Research Service. These statistics are summarized below.

<b>World Corn Trade</b>					
Thousand Metric Tons					
Date Created: 12/13/2004					
	2000/01	2001/02	2002/03	2003/04	2004/05
<b>Exports</b>	<b>December</b>				
Argentina	12,229	8,581	12,349	10,400	10,000
Brazil	3,741	3,857	3,181	5,818	3,000
Bulgaria	15	41	191	100	300
China, Peoples Republic of	7,276	8,611	15,244	7,553	4,000
EU-25	1,016	2,849	1,995	400	500
Paraguay	386	262	516	800	600
Romania	50	135	144	100	1,000
South Africa, Republic of	1,415	1,182	1,141	797	1,000
Serbia and Montenegro	50	21	200	50	600
Thailand	407	184	137	726	450
Ukraine	397	349	811	1,238	2,000
Others	1,147	1,201	1,238	1,822	1,165
Subtotal	<b>28,129</b>	<b>27,273</b>	<b>37,147</b>	<b>29,804</b>	<b>24,615</b>
United States	<b>48,329</b>	<b>47,271</b>	<b>40,924</b>	<b>48,645</b>	<b>51,000</b>
World Total	<b>76,458</b>	<b>74,544</b>	<b>78,071</b>	<b>78,449</b>	<b>75,615</b>
<b>Imports</b>					
Algeria	1,500	1,537	1,643	1,750	1,800
Belarus	169	126	43	150	150
Brazil	671	297	521	677	400
Canada	2,843	4,022	3,846	2,039	2,200
China, Peoples Republic of	89	39	29	2	200
Chile	1,362	1,278	933	1,000	1,000
Colombia	1,857	1,911	2,112	2,100	2,200
Costa Rica	513	463	514	585	600
Cuba	119	292	279	475	450
Dominican Republic	968	1,038	906	824	1,000
EU-25	3,800	3,801	4,327	5,600	2,500
Ecuador	149	309	304	475	300
Egypt	5,268	4,905	4,848	3,800	4,300
El Salvador	469	287	394	475	450
Guatemala	549	584	513	500	550
Honduras	252	217	214	230	200
Indonesia	1,280	1,149	1,633	1,350	1,300
Iran	1,265	1,261	2,157	1,700	1,900
Israel	993	1,021	776	1,400	1,000
Japan	16,340	16,395	16,863	16,781	16,800
Jamaica & Dep	221	241	218	225	225
Jordan	454	439	406	400	450
Kenya	700	20	82	100	400
Korea, Democratic Peoples Rep	688	288	144	80	200
Korea, Republic of	8,743	8,621	8,786	8,783	8,500
Lebanon	184	263	131	285	250
Libya	252	235	134	150	200
Malawi	50	86	25	150	200
Morocco	966	829	1,054	1,200	1,200
Mexico	5,928	4,076	5,269	5,707	5,800
Malaysia	2,588	2,425	2,408	2,100	2,000

	2000/01	2001/02	2002/03	2003/04	2004/05
Mozambique	40	369	413	100	200
Peru	861	858	917	1,100	1,000
Panama	249	237	272	300	300
Zimbabwe	50	311	625	600	600
Russian Federation	150	534	99	500	600
Saudi Arabia	1,389	1,268	1,424	1,550	1,600
South Africa, Republic of	0	726	617	495	200
Syria	794	892	919	950	1,100
Tunisia	776	793	734	800	800
Turkey	608	1,193	1,475	1,050	900
Taiwan	4,924	4,661	4,681	4,900	4,700
Venezuela	1,207	515	675	680	800
Yemen	200	264	252	200	200
Others	2,872	2,203	2,166	2,022	1,790
Subtotal	<b>75,350</b>	<b>73,279</b>	<b>76,781</b>	<b>76,340</b>	<b>73,515</b>
Unaccounted	<b>929</b>	<b>1,063</b>	<b>916</b>	<b>1,768</b>	<b>1,720</b>
United States	<b>179</b>	<b>202</b>	<b>374</b>	<b>341</b>	<b>380</b>
World Total	<b>76,458</b>	<b>74,544</b>	<b>78,071</b>	<b>78,449</b>	<b>75,615</b>

Source: USDA Official Estimates [http://www.fas.usda.gov/psd/complete\\_tables/GF-table7-88.htm](http://www.fas.usda.gov/psd/complete_tables/GF-table7-88.htm)

**Note:**

- The international trade year for corn is October/September.
- 'Unaccounted' includes grain in transit, reporting discrepancies in some countries, and trade to countries outside the USDA database.
- This table was prepared by the Grain and Feed Division, Commodity and Marketing Programs, Foreign Agricultural Service, USDA, Washington DC 20250. Information is gathered from official statistics of foreign governments and other foreign source materials, reports of U.S. agricultural attaches and Foreign Service officers, results of office research, and related information.

631. It is clear that the US, Argentina, People's Republic of China and Brazil are major exporters of maize.

632. From a statistical perspective, some reasonable representation of actual imports to the EU would seem necessary. There has been no attempt to do this. Indeed there seems to be a dismissive attitude to the request.

633. It can be asked if maize from Chile would in fact be representative of maize from other regions of the world. It is likely that for some industrial uses of maize, that there is little need to distinguish among regional sources. Without data supporting this lack of need to distinguish geographic sources, it is not possible to assert that Chile would be representative of all importation regions.

634. Hence, **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.**

635. Even though the question was not asked by the Panel, the scientific rationale for the request for compositional data is not evident from the written record. It would seem essential to provide such a rationale concomitant with the request for information from the notifier. More specifically, while I could imagine some reasons for such a request, many of these imagined reasons are NOT necessary for completing a scientific risk assessment.

**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?**

**Dr. Nutti**

636. Based on EC-075/At.1, regarding Question 5 on protein expression, the protein used in the toxicology and allergenicity tests was obtained in a heterologous system and not in other maize. Thus, the notifier provided the correct information, which the lead CA did not understand. The possible reason for the misunderstanding resides in the fact that the same protein is expressed in maize 1507 and in maize 1360. But, it is important to point out that the studies were carried out with protein derived from recombinant microorganisms.

637. Based on EC-075/At.1, regarding Question 6 on protein toxicity and allergenicity, the lead CA asks about products which clearly degrade during processing. The answer provided by the notifier is very well detailed and comprehensive, clarifying all the topics regarding the safety assessment of protein toxicity and allergenicity. My impression is that the lead CA was not the most suitable person to evaluate the case.

**Dr. Andow**

Necessity to ensure validity of safety assessment:

638. I will cover the toxicity and environmental impact parts of this question. I will address whether the information requested by the NBC was necessary to ensure that the conclusions of the safety assessment were valid.

639. The specific information requested by the NBC that is relevant to the part of the question I will address is detailed in EC-75/At.05, 28 November 2001, and repeated below.

**Field trials in Spain**

1. In accordance with its rules and principles, the National Biosafety Committee considers it necessary to conduct and have the results of experimental trials in Spain before proceeding to examine the application to place the product on the market or for the company to give sufficient reasons why there is no need to conduct such trials in Spain.

### **Protein expression**

5. The toxicity and allergenicity tests for *CRY1F* and *PAT* proteins must be carried out on proteins obtained from 1507 maize, not from 1360 maize which, although obtained in the same way, is a different transformation event.

6. Point 4.3(ii) on page 134 states that irrespective whether or not the process degrades the inserted sequences and the *CRY1F* and *PAT* proteins, the effects of the product on animal feed are considered insignificant since neither the *CRY1F* and *PAT* genes nor the *cry1f* and *par* proteins are toxic or allergenic. This claim is debatable, above all with regard to the possible toxicity of degradation peptides compared with full proteins. Without going any further, Bt protein itself is toxic when processed. It would therefore be desirable to check that technological processing of grain and forage derived from 1507 maize leads to no specific degradation of the proteins analysed. If it does, it would be necessary to analyse the allergenicity or toxicity of the specific degradation products.

### **Environmental impact**

9. The information provided by the company on studies is insufficient, particularly on the impact on other species and/or the environment. There are many cross-references to unpublished documents. In order to evaluate the experiments conducted, the following documents are needed: Hoxter et al. 1999 a, b, c and d; Higgins, 1999; Maggi, 1999; Halliday, 1998b.

10. Given that the Mediterranean corn borer (*Sesamia nonagrioides*) is the greatest pest in Spain, data are needed on the efficacy of this transgenic maize against this species in the laboratory and in the field (vernier 2001b).

11. Annex 1, Appendix 1 includes no non-target species of relevance to Spain. Species which are endangered, sensitive to changes in their habitat and of special interest should be covered. More specifically, species occurring at the same time or place as maize pollination must be taken into consideration.

12. The resistance management plan proposes voluntarily reserving 5% of the area as a refuge with non-Bt maize, but fails to state that insecticides must not be applied within this area so that it can serve as a true refuge. The monitoring plan must be brought into line with the legislation in force.

640. After receiving responses from the notifier, the NBC amplified their request for experimental trials in Spain by requesting additional trials, and evaluations of herbicide efficacy (EC-75/At.13, 17 June 2002). While these requests are not included in the question at hand, they suggest that request 1 in EC-75/At.05, 28 November 2001, was considered quite important by the NBC.

### **Field trials in Spain**

1. After examining the additional information supplied by Pioneer, the National Biosafety Committee repeated that more experimental trials were needed in Spain and that the results should be placed at its disposal in order to ascertain the behaviour and efficacy of this maize under the different environmental conditions which can occur

in Spain and its impact on populations of corn borer and of other non-target species associated with maize crops in Spain.

2. Data are also needed on evaluations of the efficacy of the herbicide on this crop under conditions in Spain, since this could depend on the climate in each area or region.

641. The original notification submitted by the notifier on 6 July 2001 was not included in the materials. EC-75/At.01 appears to be the original Spanish version of EC-75/At.06-11. This means that I cannot assess the necessity of the request in the context of information already provided in the original notification. Instead, I will have to judge the necessity of the requested information on the presumption that the answers provided by the notifier on 14 February 2002<sup>109</sup> to questions 5, 6, 9, 10, 11 and 12 of EC-75/At.05 were sufficient to meet the information needs requested. On this presumption, I can determine if the information provided was necessary to ensure that conclusions of the safety assessment were valid. As Question 1 apparently was not sufficiently addressed in the 14 February 2002 response, and apparently was still unresolved over a year later (EC-75/At.21, 17 February 2003), I believe too much time and additional information intervened to judge the necessity of the original question on the basis of an analysis of the response. I will evaluate this question without reference to the answers provided by the notifier.

642. Question 5. Question 5 was **necessary to ensure that conclusions of the safety assessment were valid**. It is possible that microbially derived Cry1F protein or Event 1360 derived Cry1F or PAT are not the same chemical products. If they were not the same chemical products, then results from toxicity tests could be different, leading to erroneous conclusions in the safety assessment. It is possible that identical gene sequences in the plasmid vector could give rise to different gene sequences incorporated in the plant. This has been seen for the cry1Ab gene that was incorporated into Mon810 and Bt11.<sup>110</sup>

643. Question 6. Question 6 was **necessary to ensure that conclusions of the safety assessment were valid**. It is possible that stable or transiently quasi-stable degradation products of Cry1F or PAT proteins could have effects, either by themselves as degradation products or in reacting with other chemicals, such as sugars, even when the parent compound does not react with other chemicals. I believe stable or quasi-stable degradation products of Cry proteins in humans have not been found, however, such products are known in insects. Indeed stable degradation products are the basis of the toxicity of some Cry proteins to some insects. Hence this possibility cannot be excluded in other non-mammalian degradation processes. The possibility of degradation products reacting to form chemicals with some adverse effect is a hypothetical possibility. However, the chemical reactions that occur in grain processing are complex and fully understood. It should also be recognized that the possibility of such effects may be small.

644. Question 9. Question 9 was **necessary to ensure that conclusions of the safety assessment were valid**. Actually a risk assessor can choose to believe the interpretation of the data provided by the notifier or to examine the data and come to an independent interpretation. The scientifically sound approach would be to examine the data and come to an independent interpretation. The question is both reasonable and essential.

645. Question 10. This question is difficult to understand. Vernier (2001b) is referenced in the question. This report, which was included in the original notification, evaluates lab and field efficacy

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<sup>109</sup> EC-75/At.01 (English).

<sup>110</sup> Essential Biosafety. [www.essentialbiosafety.com/main.php](http://www.essentialbiosafety.com/main.php).

of Event 1507 to *Sesamia nonagrioides*. The question asks for lab and field evaluations of efficacy to *S. nonagrioides*. Hence, from a literal interpretation of the question, it was **not necessary to ensure that conclusions of the safety assessment were valid**. Even if we assume that the question was requesting field data from Spain, the notifier's answer clearly states that the field data were collected in an area of southern France, and the NCB did not question this in their follow-up questions. Hence, it must be concluded that they decided also that the question was not essential, as acceptable data had already been submitted in the original notification. The only way that this question could be considered essential is if the data provided by the report by Casteñera (2001), cited in the notifier's response filled some significant gap in the Vernier (2001b) study. As neither of these reports were included in the information before the Panel, it cannot be concluded that the Casteñera (2001) report filled a crucial gap in the Vernier (2001b) study, and it cannot be concluded that the question was essential.

646. Question 11. Question 11 was **necessary to ensure that conclusions of the safety assessment were valid**. The original notification suggested that most susceptible Lepidoptera would not be adversely affected by Event 1507 because exposure levels would be low and the expected hazard was also low. This part of the risk assessment then presumed that all Lepidoptera of potential concern would not be adversely affected. The question was necessary to ensure that the specific Lepidoptera (butterflies) of special concern would not be adversely affected.

647. Question 12. There is legitimate scientific debates that bear on the necessity of this question. One perspective is that a 5% refuge is insufficient for corn borers regardless of how it is treated and that a 20% refuge is essential, but this 20% refuge can be treated with other insecticides. From this perspective, it is the area of refuge that is most important as long as the refuge produces some number of susceptible moths,<sup>111</sup> and Question 12 is not justified because its premises are not valid. The other perspective is that a 5% refuge may be sufficient if enough corn borers are produced from it.<sup>112</sup> In other words, refuges should be managed to produce as many susceptible moths as possible. From this perspective, question 12 was **necessary to ensure that conclusions of the safety assessment were valid**. In either case, discussion of various management options for the refuge is necessary.

648. Question 1. The degree that a field trial should be conducted in the actual location of concern is of considerable scientific interest and there is not yet a scientific consensus on this issue. Whether data from France and Italy can be used to inform a risk assessment in Spain in a scientifically definitive manner is difficult to answer in the abstract. In general, it has been appreciated for some time that climate constrains ecological communities and ecosystems creating similarities among them even when they are in widely disparate regions of the world. More recently, climate has been shown to similarly structure some biological communities, especially in comparisons of communities in the world's deserts. Thus, there are reasons to believe that data from France and Italy can be used to inform a risk assessment in Spain. At the same time, it has been widely appreciated that local ecological communities and ecosystems often function quite differently even from nearby superficially similar communities and ecosystems. For example, for maize entomology in the US, it is unlikely that an extension entomologist in Illinois would accept important results from Iowa, Indiana or Ohio as accurate for Illinois without doing some experiments to confirm their applicability.

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<sup>111</sup> This perspective has been most recently advanced by Ives and Andow, 2002, Evolution of resistance to *Bt* crops: Directional selection in structured environments. *Ecology Letters* 5:792-801. The basic idea is that selection is related mostly to the proportion of refuge moths that are exposed to selection on the *Bt* crops, which is proportional to the relative area of the *Bt* crops.

<sup>112</sup> This perspective has been widely suggested, but still has not been proved. The underlying metaphor is that the refuge moths dilute out resistance in the *Bt* crops, and the more refuge moths, the greater the dilution effect. More moths can be produced by producing more moths per unit of refuge or by having larger refuges.

Thus, there are reasons to believe that data from France and Italy may not be used to inform risk assessment in Spain, unless some of the critical information is confirmed to be relevant for Spain in experiments in Spain. Based on the exact wording of the question, it cannot be determined which of these two perspectives would hold in this case. In part, the reason for this indeterminacy is that the question is so general that it could be referring to almost any aspect of field experimentation. The question should be worded more specifically, both to guide the registrant in answering the question, and to be clarify the specific purpose of the question. As a consequence, while I would conclude that questions along the lines of question 1 would be necessary to ensure that conclusions of the safety assessment were valid, in this case, I must conclude that question 1 was **not necessary to ensure that conclusions of the safety assessment were valid.**

649. Summary. With respect to toxicity and environmental impacts, questions 1 and 10 were not necessary to ensure that conclusions of the safety assessment were valid, while questions 5, 6, 9, 11 and 12 were necessary to ensure that conclusions of the safety assessment were valid.

650. Following from this summary, given that the notifier had notified this product under the regulation concerning the deliberate release of this plant (cultivation), the concerns raised by the lead CA on toxicity of the proteins expressed by the inserted gene sequences were relevant to the risk assessment of this product.

651. The notifier submitted the original notification to the Spanish CA (NBC) on 6 July 2001. The NBC requested additional information on 30 October 2001 and 28 November 2001. The notifier responded to these requests on 14 February 2002. The NBC made additional requests for information on 17 June 2002. From the time of submission to the first request for information 116 days passed. From the time of submission of responses to the requests for information to the 17 June 2002 request for additional information, 92 days passed. This was 208 days total time, and seems longer than necessary to complete the reviews and make requests for information.

**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?**

**Dr. Nutti**

652. Based on EC-76/At.1-2 and 27, and regarding nutritional analysis, compositional analysis of NK603 maize grain has demonstrated substantial equivalence with traditional maize. This is supported by lack of differences shown in the results of feeding NK603 to broiler chickens compared with birds fed with NK603 parental line or with five commercial reference lines, as well as repeated dose feeding studies in rats, which contribute to determine the composition and nutritional equivalence. Whatever studies were further requested by the lead CA in EC-76/At.6, such studies were not necessary to ensure that conclusions of the safety assessment were valid since all the relevant information had already been provided. In EC-76/At.27, the Spanish Biosafety Commission had already estimated that for the uses considered and at the current scientific and technical "state of the art", there was no scientific evidences which indicated any risk for human and animal health of NK603 maize.

**Dr. Andow**

Necessity to ensure validity of safety assessment:

653. I will cover the environmental impact aspects of questions 37 and 38.

654. The relevant questions from the lead CA are in EC-76/At.5, 15 February 2001 and are reproduced below.

**Environmental impact of the GMO**

Although this is not the direct purpose of this notification, the application covers aspects relating to the imminent request for authorization for cultivation in another Member State. In view of this eventuality, the National Biosafety Committee raises the following questions:

16. Page 14, Section 3 – Survivability: The comments on plant survival are incorrect, given that crop repetition is common in many areas and that, in many cases, grain from fallen ears germinates to produce plants in the next season.

17. Page 16, Section 7 – Potential interactions: This point must be looked into as broadly and deeply as necessary. The environmental impact of these interactions on target or non-target flora and fauna must be evaluated more thoroughly.

18. Page 58, Point D.6. – Transferability of genetic material from the genetically modified plant to other organisms: Although the application refers exclusively to authorization of the grain, detailed knowledge of the dispersal capacity of the pollen under various conditions is necessary and would be of relevance for authorization of cultivation in the State concerned by the application. This information could be decisive when the time comes to draw up a future monitoring plan.

19. Page 98 – Appendices: Doses and conditions for application of the herbicide (phenological condition of the plant, dose and date).

655. It is clear from the opening paragraph of these questions that the lead CA justifies the questions on the basis of an imminent request for authorization for cultivation in another Member State. Thus, I conclude that the lead-CA does not believe that the questions on environmental impact are necessary to ensure that conclusions of the safety assessment were valid.

656. I believe that **none of the questions posed under environmental impact are necessary to ensure that conclusions of the safety assessment were valid.**

657. Given that the notification is not for cultivation, whether survival is slightly better in continuous maize than in rotated maize ignores the bigger point, which is that maize does not survive very well. While the CA is correct in noting the difference, this difference is not necessary to ensure that conclusions of the safety assessment were valid.

658. While it is true that potential interactions need to be looked into broadly and deeply if the GM crop were to be cultivated, it is not true that such an investigation is necessary for the present notification. It would be better to focus attention on detecting accidental releases and quickly eliminating them.

659. Although it is true that detailed information on transferability of genetic material from the GM crop to other organisms is needed if the GM crop were to be cultivated, it is not true that detailed information is needed for the present notification. Some information is necessary to consider how gene escape can occur either during processing, storage or transport, but detailed information is not necessary.

660. As the GM plant is not to be cultivated under the present notification, information on herbicide application is not needed.

661. From the time that the notification was received (2 January 2001) to the time that the lead CA sent questions for clarification to the notifier (15 February 2001), 44 days had elapsed. This seems to be a rapid turn around time.

### **Dr. Squire**

662. The weakness of suitable criteria is again at issue here (Notes, paragraphs 2, 7, 8). Documentation from the company is least on environmental matters, partly because of changed context (what might be an acceptable impact in one part of the world is considered unacceptable in another), while the counter-arguments take no step towards indicating what standards might be applied.

**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?**

### **Dr. Andow**

663. Having received answers from the notifier (5 September 2001), the lead CA requested additional information. On the issue of environmental impacts, the lead CA asked the following question.

- Although cultivation of this maize is not covered by this application for marketing authorisation, details of the potential environmental impact of any accidental dissemination or germination are needed.

664. Based on the SNIF (EC-76/At.2, 4 August 2000) and the responses to the first set of questions from the lead CA (EC-76/At.7-8, 5 September 2001), it is clear that the notifier has not addressed this question. The notifier believes, probably rightly, that the likelihood of accidental dissemination and germination (exposure to the environment) is small. If this is true, the notifier is arguing that when exposure is small, risk is small. Consequently, the notifier may believe that it was not necessary to address this question.

665. In either event, the lead CA may reason as follows: if the hazard associated with a rare exposure event is large, then the risk may be large. Hence the **question is necessary to ensure that conclusions of the safety assessment are valid**. However, it would be helpful to the notifier to specify that the lead CA is concerned about large potential environmental impacts. Moreover, it would be even more helpful to the notifier to suggest some possibilities. For example, if contamination of conventional production (related to the "coexistence" issue) is a major concern (and

is considered an environmental impact), the notifier would be able to propose how the concern could be managed, thereby facilitating the more rapid completion of the notification process.

666. The time from the responses to the first set of questions from the lead CA (EC-76/At.7-8, 5 September 2001) to the time of the second set of questions from the lead CA (EC-76/At.10, 10 October 2001) was 35 days. This seems to be a rapid turn around time.

**Dr. Squire**

667. 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.

**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?**

**Dr. Nutti**

668. Based on EC-76/At.11-12 and the request by the lead CA in EC-76/At.14, regarding to animal feeding, asking for clarification of the titles of tables 2 and 3 on page 24 of Appendix 2 and of their content with regard to the calculation of the safety margins established and the reasons for the differences detected, my opinion is that such a request was not necessary to ensure that conclusions of the safety assessment were valid.

**Dr. Squire**

669. See answer to question 37.

**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?**

**Dr. Nutti**

670. Answer 39 bis: Based on EC-76/At.11-12 and the request by the lead CA in EC-76/At.44, regarding to allergenicity studies and PCR tests, **my opinion is that such a request was not necessary to ensure that conclusions of the safety assessment were valid.**

**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?**

**Dr. Nutti**

671. Based on the information provided by the applicant (EC-85/At.25-56) and responses to the questions raised by Denmark (EC-85/At.41 and EC-85/At.32, respectively), regarding animal feeding studies, my understanding is that the applicant has provided sufficient relevant information. According to the Codex Alimentarius Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003, paragraph 53), 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.

**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?

**Dr. Nutti**

672. **Answer 40 bis:** Based on 78/85/At. 19 and the request by Denmark, Austria and Italy (EC-78/85 /At. 67, 40 and 72, asking for a second animal whole feeding study, **my opinion is that such a request was not necessary to ensure that conclusions of the safety assessment were valid.** Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability 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.

**Pioneer/Dupont high-oleic soybean (260-05)**  
**C/NL/98/09 (EC chronology 87)**

**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?

**Dr. Nutti**

673. Based on the notification (EC-87/At.1), the additional information regarding data on the composition of high-oleic soybeans (lectin content and composition data from at least two seasons.) and the quantification of the change in the protein profile of this product requested by the lead CA (EC-87/At.11 and 12) were necessary to ensure that conclusions of the safety assessment were valid.

674. It is important to point out that the lead CA has recognized that the soya oil with the changed fatty acid composition is mainly intended for human use, and not for livestock feed. It is therefore not expected that these changes will have an impact on livestock feed safety.

**Dr. Squire**

675. Response on environmental risk. This product is intended for import for processing, not for growing as a crop. Its potential for environmental risk – compared to other soybean – is through any difference in the waste the processing generates or any difference in the rate of establishment as a casual or weedy plant. It would seem that the differences in chemical composition of the high-oleic soybean are unlikely to generate a different type of waste after processing.

676. The company indicate the product does not pose a risk through becoming established as a plant "since the transgenic soybeans do not differ in their characteristics regarding survival, multiplication and dissemination from commodity soybeans" (paragraph 42 in EC-87 At-1). While there are no data presented explicitly on this point, the assertion is nevertheless well founded. In Europe, the soya-bean (soybean) *Glycine max* (L.), exists as an uncommon casual plant, mostly on tips and waste ground, originating from imported oil-seed (spilled or dumped), food-refuse and bird-seed. It is very much less common than plants arising from, say, oilseed rape: in the UK, for instance, casual soybean was recorded at less than 50 localities over many years in a major compendium of alien plants (see reference below). It does not spread, is not an agricultural weed and will not cross-pollinate with local plants. It is possible that imported high oleic soybean would come to exist as a casual plant in Europe, but there is no reason to indicate that it would exist in greater numbers, for longer or at more locations than other soybean. The high-oleic soybean does not have properties that would cause it to differ markedly in nutritional or toxic properties, and so affect insects or other wild animals, and the evidence and arguments presented about the extremely low risks regarding horizontal gene transfer (e.g. to microorganisms) appear correct. Therefore the conclusion of the Commission of Genetic Modification that the product did not present environmental risk is sound. The requests for further information (EC-87, At 11, 13, 15) appear to be mainly about safety as a feed, so the additional year's field data (requested) would not provide substantive new information on the plant's environmental risk.

Clement EL, Foster MC (1994) Alien plants of the British Isles. Botanical Society of the British Isles, London.

**Monsanto/Syngenta Roundup Ready sugar beet**  
**C/BE/99/01 (EC chronology 88)**

**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?**

**Dr. Nutti**

677. Based on the notification (EC-88 At.1) and additional information provided by the notifier (EC-88 At.11-12), my understanding is:

678. EC- 88 At.11\_SCI, pg 7, Question 3.1, asks more information on allergenicity. The additional answer is provided at Section 4, pg 13 of EC-88 At 11, with a very detailed explanation on the allergenicity assessment. The notifiers provided again the references explaining that there are two common characteristics snared 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. Assessment of this two characteristics along with careful review of the history of exposure and search for possible sequence similarity between the GM protein and known allergens can help minimize the risk of transferring a known allergen, one that is possibly going to be cross-reactive to a known allergen, or one that could have a high likelihood of being a food allergen. On pg 13 and 14, the notifiers explained the rationale for the test carried out, the decision three used for the allergenic potential proteins encoded by genes transferred to genetically modified crops, derived from FAO/WHO 1996, ILSI 1996. Although this references and this process were carried out in 1998, it is very important to point out that the allergenicity assessment was carried out according to the rules that would be established by 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 My understanding in this case is that the necessary information was provided by the notifiers in the notification, and the additional information, although was not needed, provided clarification to the lead CA, to the understanding of the allergenicity assessment.

679. EC-88 At.11\_SCI, pg 7, Question 3.2: "Can the notifying firms show that the concentration of GUS protein in the gastrointestinal tract that appears in people and animals after ingesting food products is sufficient protection against any toxicity caused by the deglucuronidation of endogenous or exogenous products?", is properly answered by the notifiers, when they explain that since sugar is derived from sugar beet and there is very little if any residual protein, there would be no meaningful exposure to GUS protein with the consumption of refined sugar. In my opinion this information was already in the notification, the additional answer was just an explanation to the lead CA.

680. EC-88 At.11\_SCI, pg 7-9, Question 3.3: "On page 64, proteins wich are toxic via acute mechanisms needs justification. The same is true of the acute toxicity test". With this question, the lead CA asks for justification for the acute gavage study tests done with mice (provided in the notification) and information why this tests has been carried out with proteins that are toxic. The notifier answered the question, explaining that acute gavage tests are usually performed with proteins that are toxic via acute mechanisms, provided the reference (EPA and Slojab at al, 1992), and explained the rationale for using this test with CP4EPSPS proteins. The notifier also reminded that those proteins were rapid degraded in in vitro digestibility studies, providing more information with new versions of paragraphs C.2.a (ii), C.2.b (ii), C.2.c (iii) in the Application, in the Appendix of Section 4 of the document. My conclusion, as far as the toxicity of the protein is concerned, is that the notifier provided the information requested and logical justification why those tests were carried out, so the information provided at EC- 80 At 11 complemented and explained the data submitted in the notification ( At 1), clarifying the lead CA, but it is important to point out that the necessary information on the toxicity of the protein has been presented at the notification, so further information was not necessary to ensure the conclusions of the safety assessment in this topic (toxicity of the protein), but it helped very much to understand all the issue on toxicity, as the answer was detailed and explained not only the tests which were carried out, but also the specific target tissue, measurements of weight and food consumption, low toxicity of the GUS protein, and minor pathological changes observed at the necropsy, so with the answer provided at AT.11, my understanding is that no more information was needed in this topic.

**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?**

**Dr. Nutti**

681. The information required by the lead CA (EC-88 At. 27-28) for allergenicity was not necessary to ensure that the conclusions of the safety assessment were valid. The previous information 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.

**Monsanto MaisGard Roundup Ready (MON 810 & GA21) corn (stack)**  
**C/ES/99/02 (EC chronology 94)**

**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?**

**Dr. Nutti**

682. Concerning the information requested by Netherlands in EC-94/At.12, I will answer the questions related to my background.

683. Question 3 – This information is not necessary to ensure the validity of the safety assessment. The lead CA was asking for more explanation about the higher level of m EPSPS protein in MON 810xGA21 maize line, as the Committee did not accept that they are due to biological variety. My opinion is that the explanation given by the notifier was acceptable, since in EC-94/ At 13, the notifier presents the results of second year study in order to test this assumption, and the results confirmed that the expression levels were similar.

684. Question 4 – the Committee asked to be provided data to confirm that glyphosate treatment has no effect on the composition of maize plants used and justification for the statistical analysis presented at annex 2 of the dossier. It was also pointed out that regarding question 3, the glyphosate treatment was not considered to be a possible factor that might influence the level of expression of the introduced trait. My understanding is that for the comparison of a GM with a non GM product, usually the treatment with herbicide is not required, as the tests are done in order to compare the effect of the new trait, and not the effect of the herbicide. In this case, the applicants had provided data with glyphosate treated and non-treated product, so the information asked was not necessary to ensure the safety assessment. This was confirmed at the answer provided by the applicant at EC-94/ At 13, where references were provided that the treatment with glyphosate does not affect the composition of GM maize or GM soybean.

685. Question 5 – the Committee recognized that data was provided on the comparison of MON810x GA21 and the parental lines MON 810 and GA 21 for grain and forage, and asked for more information, including a comparison of the new hybrid with non-transgenic parental lines, as the comparison presented was with the transgenic parental lines. The Committee asked for information on the composition of ferulic acid, p-coumaric acid, raffinose and inositol, and in this case the Committee accepts the comparison between the new hybrid with the parental transgenic lines. My understanding is that there are different approaches that can be used when GM traits have been combined by traditional breeding (stacked), one is that the comparison of the new hybrid should be done with the GM parental lines, and this GM parental lines should have been compared with conventional counterpart; another is that the new hybrid should be compared with non-transgenic

parental lines. The problem with the second option is that sometimes the relevant parental lines may not been developed or may exist only in a genetic background which is not comparable to the stacked hybrid.

686. The applicant has chosen the first option for the comparison, as data was presented with the information of the equivalence of GA 21 and MON 810 to their nearest non-transgenic isolines, and also the new hybrid MON810xMONGA21 with the parental transgenic lines. My understanding is that the information submitted by the applicant was sufficient to ensure the validity of the safety assessment. As far as information on the composition of ferulic acid, p-coumaric acid, raffinose and inositol, were concerned, my understanding is that this supplementary information was not necessary, as the applicant had already submitted information indicating that none of the corresponding pathways have been altered by the breeding of new traits. The applicant also informed that Phytic acid has been considered an important secondary metabolite in maize and its measurement encompasses a biologically relevant form of inositol.

687. Question 6 – toxicological tests, the Committee asked for a semi chronic feeding study on mice with grain or meal from MON 810x GA21maize. Based on Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 34 to 43, Assessment of Possible Toxicity, we have at para 37 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, there are doubts on the safety of the new substance. In this case the applicant has provided in the dossier (EC-94/At.1-3), all the information usually requested for the food safety assessment, for toxicological assessment he presented safety studies on protein digestion in mammalian gastric and intestinal systems, acute gavage studies in mice, homology to known toxins and allergens and exposure to human diet. Based on Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food, that is not the case of the new hybrid MON 810x GA21 maize. My understanding is that the information submitted by the applicant was sufficient to ensure the validity of the safety assessment.

**Dr. Andow**

Necessity to ensure that conclusions were valid:

688. I will answer this question with respect to the effect of glyphosphate treatment and toxicology.

689. The relevant question from the Netherlands CA (EC-94/At.12, 17 July 2000) on the effect of the glyphosate treatment is reproduced below.

4. The field trials and block treatment. In Appendix II of the dossier, the compositional analyses of the MON 810 x GA21 maize are described. The following comparisons are made: components of MON 810 x GA21 maize/forage with components of MON 810 maize/forage (both maize lines not treated with Glyphosate) and components of MON 810 x GA21 maize/forage with components of GA21 maize/forage (both maize lines not treated with glyphosate). Then, the following statement is made: "the significant differences in component values are only relevant where the mean value of the MON 810 x GA21 maize line was either greater than or less than both parental lines". However, this assumption is only viable

if there is no effect of Glyphosate treatment on the composition of the maize lines since only half of the maize plants used for analyses (maize lines MON 810 x GA21 and GA21) are treated with Glyphosate. Therefore, the Committee would like to be provided with data to confirm that Glyphosate treatment has no effect on the composition of the maize plants used and to justify the statistical analyses and comparisons between MON 810 x GA21 maize/forage (not treated with glyphosate) and MON 810 x GA21 maize/forage (treated with glyphosate).

In relation to question 3: mEPSPS protein levels in MON 810 x GA21 maize line are compared with the levels of mEPSPS protein in the parental maize lines MON 810 and GA21. In these comparisons, the Glyphosate treatment was not considered to be a possible factor that might influence the expression levels of the introduced traits.

690. The relevant question from the Netherlands CA (EC-94/At.12, 17 July 2000) on toxicology is reproduced below.

6. The toxicological tests. Recently, it was discovered that in genetically modified soybeans parts of the vector used for the modification were scattered over the genome of the soybeans. It can not be excluded that these parts are able to form proteins. The Committee is of the opinion that other plants that are modified with the particle acceleration method also might contain some unknown parts of DNA. The presence and safety of these DNA parts, however, can not be guaranteed with the current molecular biological techniques. Therefore, the Committee finds that, beside the molecular biological characterization of the modified genome, a semi-chronic toxicity study with relevant parts or products of the plants are necessary to assess the safety of the modified plant for the consumers. In the case of the new maize hybrid MON 810 x GA21, this would mean, for example, a semi-chronic feeding study on mice with maize grain or meal from the MON 810 x GA21 maize plant.

691. The statistical analysis used is given in Appendix II, page 90 of EC-94/At.2, 29 February 2000.

Statistical analyses of the U.S. and E.U. composition data was conducted separately and combined using a mixed model analysis of variance:

$$Y_{ijk} = U + L_i + B_{ij} + T_k + e_{ijk},$$

where  $U$  = overall mean,  $L_i$  = random location effect,  $B_{ij}$  = random spray block within location effect,  $T_k$  = treatment line effect, and  $e_{ijk}$  = residual error. The component values for the RR/YG lines were compared to those of the corresponding single trait parental lines (RR and YG) to determine significant differences. SAS<sup>®</sup> software was used to generate all summary statistics and perform all analyses (SAS Institute, 1989, 1990, 1996). Report tables present p-values from SAS<sup>®</sup> as either <0.001 or the actual value truncated to three decimal places.

692. The experimental design for both the US and EU field studies was similar. There were six sites in the US trial and seven sites in the EU trial. At each site the field was split into two glyphosate treatments (plus glyphosate or no glyphosate). All of the RoundUp Ready<sup>®</sup> (RR) varieties were planted in the plus glyphosate whole plot. Those lacking RR were in the no glyphosate whole plot. Within each whole plot, the relevant YieldGard<sup>®</sup> (YG) varieties (both DeKalb and Holden parentage)

and those lacking YG were planted (presumably randomly) as the split plots within the whole plots. Thus there were four split treatments within each of the two whole plots.

693. From the description in paragraph 691, it would appear that the notifier compared RR+ versus RR- means based on estimated standard errors of the means from the stated statistical analysis.

694. In my opinion, the notifier used the wrong statistical analysis. The experiment is set up as a randomized complete block-split plot design with the following statistical model:

$$Y_{ijk} = \mu + L_i + RR_j + \varepsilon_{ij} + T_{ik(j)} + \varepsilon_{ik(j)}$$

695. where  $i$  indicates location,  $j$  the glyphosate treatment and presence/absence of the RR gene, and  $k$  a treatment level.  $Y$  is the response,  $\mu$  is the grand mean,  $RR$  is the glyphosate treatment/RR gene effect,  $\varepsilon_{ij}$  is the whole plot error for testing the  $RR$  effect,  $T$  is the treatment effect, nested within split plots, and  $\varepsilon_{ik(j)}$  is the residual error. The  $T$  effect can be decomposed into the following orthogonal effects:

$$T = YG + V + YG * V + YG * RR + V * RR + YG * V * RR$$

696. where YG is the effect of the YG gene, V is the effect of parental type (DeKalb versus Holden), and the other terms are the interaction effects. All of the  $T$  components have 1 df. As there is no true replication within locations, it is essential to treat locations as replicates or there would be no source of error variation. In doing this, it is readily recognized that locations are identical to blocks, so the whole plot design is a randomized complete block design. The appropriate ANOVA table for this analysis for the EU trial is:

LEVEL	EFFECT	df	MS	F	p
Level 1	Location ( $L$ )	6			
	$RR$	1			
	Error 1	6			
Level 2	$T$	6			
	$YG$	1			
	$V$	1			
	$YG*V$	1			
	$YG*RR$	1			
	$V*RR$	1			
	$YG*V*RR$	1			
	Error 2	36			

697. This analysis would allow direct statistical test of all relevant effects. It should be noted, however that the RR gene effect is totally confounded with the effect of the application of glyphosate in the experimental design. Thus it is not possible to estimate an RR effect independent of a glyphosate effect.

698. Moreover, the notifier treats each of the many response variables as if they were completely independent responses. Statistically it is inadvisable to treat each response independently with a series of ANOVAs (which is what the notifier did). In actuality it is likely that many of the response variables will be correlated with each other. Thus, the notifier should have considered several

possible multivariate responses, and conducted several intelligently planned MANOVAs prior to the many ANOVAs. Statistically it is inadvisable to use all of the data in a single large MANOVA.

699. Thus the CA was justified to request additional information on the glyphosate treatment, the statistical analysis, and the comparisons made in Appendix II. These were necessary to ensure that conclusions of the safety assessment were valid.

700. With respect to the toxicity question, the NL-CA is correct to suggest that there may be parts of the vector scattered over the genome. However, with respect to maize, it should be possible to eliminate most of these via the backcrossing methods used to transfer a transgene into a commercializable inbred line. The only ones that are likely to remain are ones that are closely linked to the transgene locus. It would seem that the notifier would have other alternatives to the toxicity test to address the concern of the Netherlands CA. By providing details such as the number of backcross generations, it would be possible to calculate a probability that vector parts remain scattered around the genome. Soybean, being predominantly selfing would not readily eliminate such vector parts from its genome during the plant breeding process. Thus, while the concern is valid and needs to be addressed to ensure that the conclusions of the safety assessment are valid, the request for toxicity testing is not necessary to ensure that the conclusions of the safety assessment were valid for maize.

701. The notifier submitted the original notification to the NL-CA on 29 February 2000. The NL-CA requested additional information on 17 July 2000. A 139 day interval elapsed. This seems to be a little longer than necessary to complete the review and request additional information.

**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?**

**Dr. Nutti**

702. Answer 44bis: Based on (EC 94/At.3, pp. 40), and the request by the lead CA (EC 94/At.12), asking for a second animal whole feeding study in order to identify potential adverse effects, **my opinion is that such a request was not necessary to ensure that conclusions of the safety assessment were valid.**

703. Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability 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 MON 810 & GA21.

**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?**

**Dr. Nutti**

704. Answer 44ter: As I already pointed out in my answer of question 44, my understanding is that there are different approaches that can be used when GM traits have been combined by traditional

breeding (stacked), one is that the comparison of the new hybrid should be done with the GM parental lines, and this GM parental lines should have been compared with conventional counterpart; another is that the new hybrid should be compared with non-transgenic parental lines. The problem with the second option is that sometimes the relevant parental lines may not been developed or may exist only in a genetic background which is not comparable to the stacked hybrid. The applicant has chosen the first option for the comparison, as data was presented with the information of the equivalence of GA 21 and MON 810 to their nearest non-transgenic isolines, and also the new hybrid MON810xMONGA21 with the parental transgenic lines. **My understanding is that the information submitted by the applicant was sufficient to ensure the validity of the safety assessment.** In this case I believe that given the results of the risk assessment studies of the biotech parents, **no** additional studies were necessary or useful to ensure that the conclusions of the safety assessment related to the hybrid plants were valid.

### **Novel foods**

#### **Monsanto Roundup Ready corn (GA 21)** **(EC chronology 91)**

**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)?**

#### **Dr. Nutti**

705. Although detection methods are not my main area of expertise, I had analysed the information presented at EC-91/At.49-56, and I noticed that at EC-91/At.50, the applicant submits not only the protocol ( methodology) used for the detection, but also comments on the main issues that should be taken into consideration, as the preparation of standard DNA solution, validation parameters, precision of measurements, accuracy, robustness, range and sensitivity.

706. EC-91/At.51, the applicant informs what are the materials that should be used in the analysis.

707. EC-91/At.52, the applicant submits more specific information on the protocol already sent and specific data for the validation of GA21 detection methodology.

708. EC-91/At.53, the applicant informs that the reagents will be sent.

709. EC-91/At.54, the applicant apologizes for the delay and informs that more reagents will be sent. Applicant asks information for the amount and type of reagent requested.

710. EC-91/At.55, JRC extends the deadline and answers the applicant about the amount and type of reagents.

711. EC-91/At.56, JRC sends the draft protocol.

712. Based on the information available, I have the impression, that there were no scientific or technical reasons which explain why the process of validating the detection method took at least fourteen months. It is important to point out that the existing method submitted by the applicant at EC-91/At.50, was complete and detailed, being already in use, so the JRC was supposed to test and validate it.

**Syngenta Bt-11 sweet corn**  
**(EC chronology 92)**

**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?**

**Dr. Nutti**

713. The methods available in 2002 were sufficient to enable to detect the DNA and the protein of Bt11 sweet corn.

714. Beginning in the 1990's, commercial protein-based and DNA-based detection methods have become available. Both methods can be used in a qualitative and a quantitative way. Early DNA-based methods have been mainly designed as qualitative methods only. More recently, there has been a shift towards quantitative PCR methods. Today, most published methods are based on real-time PCR technology that can be operated both in a qualitative and a quantitative way. Some DNA-based methods were designed for the identification of a specific event while others were designed to identify only a certain vector construct or a common genetic element (e.g., 35S promoter). Today, the development of these three different types PCR methods continues. The selection of method depends on the intended purpose. The experience for protein-based methods is similar to the experience with DNA-based methods – there are many commercially available protein-based methods also. These methods can confirm the presence or absence of specific proteins expressed by the different biotech events. These protein-based methods can be applied in both a quantitative and qualitative fashion. The protein based methods, while not event-specific, can identify uniquely most commercial events.

**Dr. Healy**

715. **Response:** In 1998, the SCP expressed a favourable opinion about the safety and human health impacts of the field maize line Bt-11. Subsequently the European Commission published its decision to grant consent to the marketing of the Bt-11 line of maize, but not the cultivation of this line. In November 1998, Novartis submitted to the Dutch authorities a request for an authorisation of food products derived from fresh and processed sweet corn carrying the Bt-11 transformation event. This request was subsequently sent to the European Commission and distributed to the Member States, some of which raised issues of concern. The Scientific Committee for Food (SCF) reviewed the scientific information underpinning the request for authorisation and expressed the view that 'Bt-11 sweet maize is as safe for human food use as its conventional counterparts'.

**Background:**

716. The Bt-11 corn line contains a single copy of two transgenes, namely the cry1A(b) and pat genes. The cry1A(b) gene encodes one of the Bt toxins derived from the common bacterium *Bacillus thuringiensis*. The toxins are selectively active against groups of insects, particularly moths and butterflies, beetles, flies and mosquitos. The Cry1A(b) protein toxin is selectively active against lepidopterans (ie moths and butterflies). The pat gene is derived from a common soil bacterium and encodes the enzyme phosphinothricin acetyl transferase (PAT), which inactivates the herbicide glufosinate ammonium and confers herbicide tolerance to the genetically modified corn.

717. No other marker genes, including antibiotic resistance genes were transferred, to Bt-11 corn.

Detection methods:

718. Methods to detect transgene(s) and their product(s) may be based on either the detection of specific DNA sequences (Polymersase Chain Reaction; PRC) or on the identification of the proteins produced by the transgene(s). The documents submitted by Novaris/Syngenta to support the approval of products derived from Bt-11 reference several DNA based detection methods for Bt-11 corn lines. These include the method of Zimmermann et al (2000), based on quantitative polymerase PCR at the integration site of the transgene, a PCR based method subjected to pre-validation studies by the Joint Research Centre (EC 92- 59; it is not clear from the available documents if this method is also based on the method of Zimmermann et al, 2000) and another method developed by Syngenta in conjunction with the Joint Research Centre and subjected to validation procedures (EC 92-60, 63, 64, 65 and 66; Rolling et al 2003). However, it appears that none of these DNA based procedures were available commercially in 2002 (Codex Intergovernmental Taskforce on Foods Derived from Biotechnology, 2002; Bonfini et al, 2002).

719. Methods for the detection of the transgenic proteins expressed by Bt-11, as raised in question 46, could focus on either of the PAT or the Cry1A(b) proteins, as the genes encoding both proteins have been transferred to Bt-11 and both are expressed in Bt-11 plants. The availability of detection methods for the detection of the PAT protein are discussed under question 17; the discussion here focuses on the detection of the Cry1A(b) protein.

720. A method based on the detection of the Cry1A(b) protein by an antibody was available in 2002 (Intergovernmental Taskforce on Foods Derived from Biotechnology, 2002). This method had been developed to quantitatively determine the levels of Cry1A(b) in the transgenic corn line MON810. The method was subjected to a large inter-laboratory validation trial to test the performance of a kit based on the method and used corn flour samples as the test material (Bonfini et al, 2002).

721. While the test method was designed to quantify Cry1A(b) in MON810 plants and their derivative food products, the method should be able to detect Cry1A(b) in any plant variety in which this protein is expressed in sufficiently high levels. It has been demonstrated that the method does indeed detect the Cry1A(b) from Bt-11 in samples containing as little as 0.1% Cry1A(b) (Stave, 2002).

722. It should be noted that the protein based detection method for Bt-11 does not specifically detect Bt-11 plants and their food products. Rather it detects any plant and food product that contains Cry1A(b) protein at detectable levels – a number of crops have been genetically modified with the gene encoding Cry1A(b). This is a general limitation of protein based detection methods. Furthermore, the method is only functional for those foods derived from plant tissue that express the protein and accurate quantification is not always possible (Stave, 2002).

723. It is also notable that both DNA and protein based methods are effective in foods only if the DNA or protein is retained intact through any processing (eg DNA and proteins are generally removed in oil extraction and refinement processes) and can be extracted and purified from food matrices.

Conclusion:

724. The available evidence indicates that an immunoassay based detection method suitable for the detection of Cry1A(b) protein in Bt-11 transgenic plants was commercially available in 2002.<sup>113</sup>

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**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)? 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?**

**Dr. Nutti**

725. Although detection methods are not my main area of expertise, I had analysed the information presented at EC-92/At.54-56, 57-65, and 66 and I noticed that the applicant submitted the material and proposed methodology, JRC tested the method and at EC-92/At.56, JRC points out some problems that were found, mainly with the amount of DNA detected in some samples, asks for comments and more material for testing.

726. EC-92/At.57, JRC explains to the applicant the main issues discussed by phone and acknowledge the reception of the material for re-starting the validation of the tests.

727. EC-92/At.58, the applicant submits more information on the methodology.

728. EC-92/At.59, confidential report from JRC, informing that the method submitted by the applicant could not be accepted for a full validation before a final method optimization is carried out, pointing out problems on the amplification of the GM specific system. JRC informs that they are willing to discuss the problems with the applicant and share expertise.

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Zimmermann, A., Luthy, J, Pauli,U. (2000). Event Specific transgene Detection in Bt-11 Corn by Quantitative PCR at the Integration Site. *Lebensm.-Wiss.u.-Technol.* 33: 210-216.

729. EC-92/At.61-65, several e-mails are exchanged between the applicant and JRC, about the methodology validation.

730. EC-92/At.66, JRC informs the conclusion of the validation and acceptance of the method.

731. Based on the information available, I have the impression, that there were no scientific or technical reasons which explain why the process of validating the detection method took twelve months.

732. I don't have expertise or references to answer about the adequate amount of material to be used in the detection method validation and adequate performance indicators for pre-validation results for this product.

**Bayer LibertyLink soybeans**  
**(EC chronology 93)**

**Question 48: Given the information before the Panel, what type of data should be used to evaluate substantial equivalence of these soybeans given their intended end-use (human consumption)? Was information regarding substantial equivalence, presence of PAT DNA and pat protein requested by the Belgian Biosafety Council (EC-93/At.11) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

733. Based on the information provided by the applicant (EC-093/At. 01-2) and the request for additional information by the Belgian Biosafety Council (EC-93/At.11), as far as substantial equivalence is concerned, my understanding is that although the notifier provided a good comparative analysis of two transgenic lines and one non-transgenic line, with information on a large number of nutrients and anti-nutrients, the request for data on composition (soluble and insoluble nutritional fibres, vitamin, mineral and trace elements contents) was proper since this information is relevant for the comparison. This is due to the fact that, when a product is a source of fibre, as in this case, the comparison should take into account its soluble and insoluble fibres contents. Besides, products derived from soybean are considered to be a good source of vitamins and minerals.

734. The request for the company to present information on the presence of PAT DNA and PAT protein in derived soya products is also correct, as the notifier presented only data on the protein content in the seeds and derived products, but did not present any specific information on PAT protein.

735. My understanding is that the requests were necessary to ensure that the conclusions of the safety assessment were valid.

**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?**

**Dr. Nutti**

736. Based on the application (EC-93/At.1-2), the request from the Greek authorities (EC-93/At.16), items a and b, regarding to the information on the analytical methods used for

determination of anti-nutrients and data on the nutritional and composition parameters of the transgenic and non-transgenic products, is correct, as I could not find this necessary information in the dossier. Based on the application (EC-93/At.1-2), concerning the request from the Italian authorities (EC-93/At.17), my understanding is:

737. Questions 1 and 2, related to the herbicide treatment in the tests and possible residues of this treatment in the final product. My understanding is that the risk assessment conducted here is related to the GMO and not to the herbicide, so this information is not relevant for the conclusions of the safety assessment. It is important to point out that the herbicide residue in the product has to be within the limits established by JEFCA and the Codex Alimentarius, so the herbicide has been assessed in a different study. Sometimes, companies that are carrying out the safety assessment of herbicide resistant GMOs, perform field tests with and without herbicides, but this is not the main objective of the evaluation, as we need to compare the transgenic and non transgenic crops and not the utilization of the herbicide.

738. Question 3, asking detailed information on the analytical methods used to evaluate composition in macro and micro nutrients and the content of anti-nutritional factors, is correct, as I could not find this necessary information in the dossier.

739. Question 4, about accreditation status of the laboratory that carried out the composition analysis, is correct, as according to the Codex Guidelines the analysis should be performed under Good Laboratory Practices.

740. Question 5, asking the study of the acute toxicity of the PAT protein to be presented, is correct, as I could not find this necessary information in the dossier.

741. Question 7, asking data on substantial equivalence to be presented on composition or animal tests carried out by the company, is correct, as I could not find this necessary information in the dossier.

742. Remark on the necessity of carrying out a study of sub-acute toxicity to obtain a more detailed assessment of toxicological aspects. In my opinion, if the information on the equivalence were properly supplied and an acute toxicity gavage study was carried out with satisfactory result, it would be not necessary to carry out a sub-acute test. As the notification does not present all the necessary information on equivalence, I cannot judge this request.

**Question 50: Given the information before the Panel, including the application and the information provided by Aventis Crop Science (EC-93/At.21), was additional information regarding nutritional composition requested by Belgium (EC-93/At.23) necessary to ensure that conclusions of the safety assessment were valid? Does any scientific evidence exist to demonstrate that genetically-modified soybean varieties are toxic to humans or animals? If so, what risk management options exist to mitigate any such risks?**

**Dr. Nutti**

743. Looking at EC-93, At.21 and 22, I understand that the notifier has provided information with four additional data packages on nutritional composition of Liberty Link Soybean A2704-12 and A5547-127. I could not find these additional information in the files that were sent to me, as At .21. At 22 are only the letters containing the additional information.

744. In EC-93/At.23, Belgium asks again for the data on neutral and acid detergent fibre, and as stated before, I agree that this is information necessary for the comparison among the transgenic and non-transgenic lines. So, I agree with this request but I cannot find the data packages on nutrition composition. Therefore, if the notifiers had provided the requested information on fibre, the compositional comparison could be concluded, but it seems that the lead CA could not find these data in the additional packages.

745. As far as the broiler chicken study is concerned, at the original dossier, EC-93/At.2. pg 29, it is described that these studies were conducted at the University of Guelph (Leeson 1997 and 1998), but I did not find a copy of the study in the dossier or in the supplementary information, so I don't know if the lead CA has the study or not. My understanding is that this specific information is relevant for the risk assessment, and the dossier informs that the results showed no difference, although they are not presented. The scientific evidence provided was not sufficient to demonstrate that the genetic modified soybean is toxic to humans or animals. The problem was that the information was not complete in order to fulfil all the steps for the risk assessment. So, before concluding that the product was toxic, the requested information was necessary to conclude the safety evaluation.

746. I would not go into risk management options to mitigate risks, if I am not sure if such risks exist. Therefore, my conclusion is that the lead CA could not conclude about the toxicity of the product or about the nutritional and compositional equivalence either, so it could not be stated that the product might be safe or toxic.

**Pioneer/Dow AgroSciences Bt corn Cry1F (1507)**  
**(EC chronology 95)**

**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?**

**Dr. Nutti**

747. Based on the application presented in EC-95/At.1-2 and the notifiers' answers in EC-95/At.10-12, I will comment on the compositional analysis and the toxicological analysis requested by Netherlands (EC-95/At.8 and 13):

748. Studies for the analysis of nutrient composition of grain were carried out in Chile (1998 and 1999), France and Spain (1999), comparing the GMO and the conventional crop, and also the use of the herbicide, concluding that 1507 maize is comparable and nutritionally equivalent to grain from existing commercial maize hybrids and spraying with glufosinate ammonium does not have an effect on the nutrient composition of the 1507 maize grain. The lead CA asked for 3 seasons of field tests in order to compare the results, but generally 2 seasons are acceptable. My understanding is that the notifier has provided 2 seasons in Chile and one season in France and Spain. I also understand that the locations in Chile, Spain and France are a representative set of locations. In EC-95/At.10-12, the notifier explains the seasons and informs about other tests carried out in France, Italy and Bulgaria in 2000, so my understanding is that the notifier has undertaken different representative places for more than 2 seasons so and, therefore, the additional request was not necessary.

749. The lead CA requested that the compositional data should be complemented with levels of Fe, Cu, Se, Zn, Vitamin A, vitamin B6 and nicotinic acid. At In EC-95/At.11, pages 10 to 12, the notifier supplied the requested data on minerals and Vitamin A, vitamin B6 and nicotinic acid. So, my understanding is that this additional information covered the necessary items for the nutrient comparison, concluding that the GM and non GM crop were equivalent. My understanding is that the additional request was necessary and it was covered with the supplementary information.

750. Data on the broiler chicken study was also supplied in At EC-95/At.11, annex 4 of the application. So, the additional request was not necessary.

751. The Committee asked the original papers of Brooks (2000), Evans (1998), Glatt(1999) Herman (2000), Kuhn (1998), Meyer (1999) and Pfister (1996), which were not included in the dossier. The lead CA also pointed out the importance of the equivalence of the novel proteins expressed in the 1507 maize and the proteins used in the toxicological tests. A 90-day oral toxicity study with maize grain or meal on mice was required in order to provide additional data that no observed negative effects on food safety would result from a possible unintended change in the 1507 maize. My understanding is that this additional request was necessary and it was covered with the supplementary information as in EC-95/At.11, the applicant presented all the requested papers, and information regarding the equivalence of the proteins (page 13), so this information was properly provided. As far as the 90-day oral toxicity study with maize grains in rats is concerned, the applicant informed that this study was under way and it would be submitted to the Committee by July 2002. Furthermore, according to the Codex Alimentarius Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003, paragraph 53), additional animal 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).

**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 Gezondheidsraad (EC-95/At.15) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

752. My understanding of the request in EC-95/At.15 is that the Committee discussed the weight of evidence and all information and concluded that sufficient data on the CRY1F protein was therefore available, substantiating the food safety of the 1507 maize regarding the presence of this novel protein. So far, as my knowledge goes, the information submitted for allergenicity was enough and there was no need for providing additional information on putative fusion proteins from the flanking regions of the insert in 1507 maize.

**Monsanto Roundup Ready corn (NK603)**  
**(EC chronology 96)**

**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 Gezondheidsraad (EC-96/At.7) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

753. Based on the application presented in EC-96/At.1-2, I will comment on the compositional analysis and toxicological analysis requested by Gezondheidsraad (EC-95/At.7):

754. Question 2, concerning the request for a semi chronic toxicity study in mice or rats, using maize grain or meal, in order to rule out possible undesired effects of additional, unidentified changes. Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 34 to 43, Assessment of Possible Toxicity, we have at para 37 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, there are doubts on the safety of the new substance. In this case the applicant has provided in the dossier (EC-96/At.1-2) all the information usually requested for the food safety assessment, for toxicological assessment he presented safety studies on CP4EPSPS protein digestion in mammalian gastric and intestinal systems, acute gavage studies in mice, homology to known toxins and allergens and exposure to human diet. It was also confirmed that the GM maize was equivalent in composition and nutrition to the conventional counterpart. Therefore, in my opinion, there was no need for requesting a semi chronic toxicity study in mice or rats, using maize grain or meal, in order to rule out possible undesired effects of additional, unidentified changes. Furthermore, according to the Codex Alimentarius Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003, paragraph 53), additional animal testing will be required if the composition is not comparable to conventional foods, which is not the case of Monsanto Roundup Ready corn (NK603).

755. Question 3 asked clarification on the data of the field trials presented in appendix II-IV of the application. My understanding is that the information was there and they were just asking for the exact description of the tests and the statistical design.

**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?**

**Dr. Nutti**

756. Answer 53bis: Based on (EC 96/At.2, pp. 102-103), and the request by the lead CA (EC 96/At. 7) asking for a second animal whole feeding study in order to identify potential adverse effects, **my opinion is that such a request was not necessary to ensure that conclusions of the safety assessment were valid.**

757. Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the

bioavailability 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 NK603.

**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?**

**Dr. Nutti**

758. Based on Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food. So, the request from Italy for more animal feeding studies was not necessary to ensure that conclusions of the safety assessment were valid. My understanding is that the dossier presented followed not only the OECD consensus document but also the Codex guidelines, as far as nutrition, composition and toxicology is concerned. It is clear for me that the substantial equivalence studies compared the GM with the conventional counterpart. Therefore, my opinion is that the Italian request on these topics was not necessary to ensure that conclusions on the safety assessment were valid. I can not answer on the questions related to molecular characterization (a to h).

**Bejo-Zaden Transgenic Radicchio rosso**  
*(EC chronology 97)*

**Question 55: Given the information before the Panel, including the application (EC-97/At.1), and additional information provided by the notifier (EC-97/At.25-28), was additional information requested by the SCF (EC-97/At.20, 22-24, 28, 31, and 30), regarding molecular characterisation, antibiotic resistant marker genes, and substantial equivalence necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

759. Based on the information presented in EC-97/At.1, 25 and 28, I will comment on the substantial equivalence item requested by SCF. The notifier has conducted several field tests from 1994 to 1996, where different characteristics were observed (colour, size of the plants, productivity etc). The notifier presented some data on the composition for substantial equivalence in the first dossier. In EC97, At. 28, the SCF informs that the previous information was formally accepted and requested analysis on crude protein and amino acid composition, carotenoids, folic acid and vitamin C. My opinion is that this request is correct, since these parameters are important for the nutritional evaluation of the product.

**Bejo-Zaden Transgenic Green hearted chicory**  
*(EC chronology 98)*

**Question 56: Given the information before the Panel, including the application (EC-98/At.9) and additional information provided by the notifier (EC-98/At.35-37 and 39), was additional information requested by the SCF (EC-98/At.30, 32-33, 34, 38, 40 and 41), regarding molecular characterisation, antibiotic resistant marker genes, and substantial equivalence necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

760. Based on the information presented in EC-98/At.9, 35 and 37, I will comment on the substantial equivalence item requested by the SCF. The notifier has conducted several field tests from 1994 to 1996, where different characteristics were observed (colour, size of the plants, productivity etc). The notifier presented some data on the composition for the substantial equivalence assessment in the first dossier. In EC97, At. 33, the SCF states that substantial equivalence was claimed on the basis of a very small sample under conditions that have not been clearly described and asks the applicant to confirm substantial equivalence over two growing seasons and in six geographical locations. The SCF also requested care to ensure that appropriate comparators were used and that these were compared with an appropriate sample size at each location. The SCF stated that if substantial equivalence should not be established, further detailed safety examination of the crop must be undertaken. I agree with the SCF comments and request in At 33.

761. In At 35, the applicant informs that the information on substantial equivalence was given before, as information on chemical composition, taste tests, phenotypical description of breeding lines and varieties, seeds and so on and informs that the data produced on crude protein, amino acid composition, biogenic amines, carotenoids, vitamins and bitter compounds provided in may 1999 should be seen as additional to the information given before. The applicant pointed out that all this information indicates that the GM chicory is equivalent to the conventional counterpart and there is no sense in continuing with experiments for longer periods.

762. In At 38, the SCF informs that the previous information submitted by the notifier on November 2000 was formally accepted and requested analysis on crude protein and amino acid composition, carotenoids, folic acid and vitamin C. As I could not find this information on the files submitted by the notifier, my opinion is that this request is correct, as this parameters are important for the nutritional evaluation of the product.

763. My conclusion is that the request from SCF regarding substantial equivalence was necessary to ensure that conclusions of the safety assessment were valid.

**Pioneer LibertyLink and Bt (T25 x MON 810) corn (stack)**  
**(EC chronology 101)**

**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-101/At.14) necessary to ensure that conclusions of the safety assessment were valid?**

**Dr. Nutti**

764. Based on the information presented in EC-96/At.1-3, At 13, I will comment on the compositional analysis and toxicological analysis requested by The Netherlands (EC-101/At.14):

765. Question 2 is about field trials and comparison between the composition of untreated and ammonium glufosinate treated samples. In my opinion, the required information is not relevant to ensure that conclusions of the safety assessment were valid, as in At-13, as I agree with the notifiers' explanation that the aim of the safety assessment was not the ammonium glufosinate, but the new product, obtained by a crossing T25xMON 810 corn.

766. Question 3, requests information on the levels of ferulic acid, p-coumaric acid, raffinose and inositol, as the Committee believes that determining the levels of these plant metabolites is important to rule out fortuitous changes in the plant metabolism as result of the genetic modification. My opinion is that this 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. I am also of the opinion 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.

767. Question 4, on toxicological tests, asks for a semi-chronic oral toxicity study on mice or rats with maize grain or meal from the hybrid lines, in order to provide additional data of the food safety of this hybrid. I would like to recall the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, that states that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food. Therefore, the request from the Netherlands for semi-chronic oral toxicity study on mice or rats with maize grain, were not necessary to ensure that conclusions of the safety assessment were valid, as the data provided by the notifiers confirmed that T25 and MON 810 are substantially equivalent to other commercial maize. In particular, it was shown the absence of expression of any other sequence or fragment of the sequence inserted in T25 and MON 810. As far as the new proteins expressed in T25 and MON 810 are concerned, for toxicological assessment were presented studies on protein digestion in mammalian gastric and intestinal systems, acute gavage studies in mice, homology to known toxins and allergens and exposure to human diet, that I understand are sufficient for the toxicological assessment. In addition, a poultry feeding study has been carried out with grain from T25xMON810, T25, MON 810 and non GM control with comparable genetics. The results presented showed that no significant differences were observed on mortality, body weight gain and feed conversion between chickens fed a diet containing maize grain from T25xMON810, T25, MON 810, the non GM control or a standard diet containing yellow dent maize. I am of the opinion that this additional study confirms that the conventionally derived T25xMON 810 maize is nutritionally equivalent to T25, MON810 and maize from non-GM commercial hybrids, so it would

not be necessary to carry out a semi-chronic oral toxicity study with maize or meal, as this study with broilers has already provided the information with the grain.

**Dr. Andow**

Necessity to ensure validity:

768. In this question, I will address field trials, secondary plant metabolites, and toxicological tests.

769. The questions from the lead CA are reproduced here (EC-101/At.14, 23 April 2001).

2. The field trials. The Committee would appreciate receiving the results of the comparison between the composition of untreated and glufosinate-ammonium treated T25 maize by Aventis, as mentioned in your letter.

3. Secondary plant metabolites. In our previous letter we requested information on the levels of ferulic acid, p-coumaric acid, raffinose and inositol. The Committee believes that determining the levels of these plant metabolites is important to rule out fortuitous changes in the plant metabolism as a result of the genetic modification. The Committee has not received this information on either the hybrid or both parental lines.

4. Toxicological tests. Although your remarks about the repeated backcrossing to the non-GM inbred line have been noted, the Committee is still convinced that a semi-chronic oral toxicity study on mice or rats with maize grain or meal from the hybrid line is required to provide additional proof of the food safety of this hybrid.

770. The necessity of question 2 on composition of treated and untreated T25 maize is difficult to determine. The comparison between untreated T25 maize and untreated maize lacking T25 would seem to provide the necessary evidence that T25 does or does not affect the composition of maize. However, as indicated by the lead CA, it is also possible that the application of the herbicide could affect the composition of T25 maize. The notifier makes a compelling point that the herbicide registration process should account for all possible adverse effects of the herbicide. However, the herbicide registration process does not require demonstration of substantial equivalence. Moreover, the herbicide is normally used with the T25 maize. Thus, **there is a scientific justification for requiring comparison of T25 with glufosinate**, T25 without glufosinate and maize without T25 and without glufosinate. However, I believe that there is a legitimate scientific debate as to whether all three are necessary to ensure that conclusions of the safety assessment were valid.

771. The necessity of information about secondary plant metabolites can be justified by the following argument. 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. The likelihood of the possibilities may be small, but it is difficult to argue how small. Secondary plant compounds include many chemicals that are human health hazards at high enough concentrations. The requested information **can be considered to guard against possible changes in human health hazard, and therefore can be considered necessary to ensure conclusions of the safety assessment were valid**. However, it is also possible that requests for information change in a way that is not entirely justified. In this case, this does not appear to be the problem.

772. My response to this question is identical to my response to question 44 in paragraph 700. This is **not necessary to ensure conclusions of the safety assessment were valid.**

773. The notifier submitted the original notification the the NL-CA on 20 April 2000. On 17 July 2000 the NL-CA requested additional information. On 22 November 2000 the notifier submitted responses to this request. On 23 April 2001 the NL-CA asked for additional information. From the time of original submission to the first request for additional information, 88 days passed. From the time of submitting responses to the second request for additional information, 152 days elapsed, including the winter holidays. This is a total of 240 days for review. This seems to be too long, even taking into account delays associated with the winter holidays.

**Dr. Squire**

774. Similar difficulties are evident in the exchanges to those in 37-39 and elsewhere! Even so, the requests by the Netherlands are arguably consistent with the type of information indicated in the *Codex Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.*

**Monsanto/Syngenta Roundup Ready sugar beet (77)**  
**(EC chronology 102)**

**Question 58: Given the information before the Panel, including the application (EC-102/At.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.**

**Dr. Nutti**

775. Based on the information submitted by application EC-102/At.1-20 and further information provided by Monsanto/Novartis (EC-102/At.22, 26 and 27-30), my understanding is that the information requested by the lead CA in EC- 102/At.32, regarding to the derived proteins and the request for a semi-chronic oral toxicity test on mice or rats with edible parts of sugar beet, was not necessary to ensure that the conclusions of the safety assessment were valid. The applicants had performed 90-day acute toxicity test with rats.

776. Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 34 to 43, Assessment of Possible Toxicity, we have at para 37 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. In this case, the applicant has provided in the dossier (EC-102/At.1-20) all the information usually requested for the food safety assessment. Also, for the toxicological assessment he presented safety studies on protein digestion in mammalian gastric and intestinal systems, acute gavage studies in mice, homology to known toxins and allergens, and exposure to human diet.

777. Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the

bioavailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food, that is not the case of Monsanto/Syngenta Roundup Ready sugar beet.

778. It was also confirmed that the GM crop was equivalent in composition and nutrition to the conventional counterpart. Therefore, in my opinion, 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.

## **ISSUE 2**

*Background: In cases where a biotech product has obtained approval for Community-wide marketing, individual EC member States may under certain circumstances provisionally prohibit the marketing of that product in their own territories. Such measures are commonly referred to as "safeguard" measures.*

*It would be useful for the Panel to understand,*

(a) *For each biotech product for which a safeguard measure was taken by specified EC member States, how the scientific or other documentation relied upon by these member States compares with:*

- (i) *the International Standard for Phytosanitary Measures (ISPM) No. 11 for Pest Risk Analysis for Quarantine Pests, including its Supplement (EC-130);*
- (ii) *FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (attached);*
- (iii) *FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (attached);*
- (iv) *FAO/WHO Codex Annex on Possible Allergenicity Assessment (attached); and*
- (v) *Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (EC-1).*

(b) *For each product for which a safeguard measure was taken by one of the specified EC member States, whether the scientific or other documentation relied upon by these member States is sufficient to support the safeguard measures taken.*

*The SPS Agreement defines risk assessment as:*

*"The evaluation of the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied, and of the associated potential biological and economic consequences; or the evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease-causing organisms in food, beverages or feedstuffs."  
(Annex A, paragraph 4)*

Furthermore, the SPS Agreement provides that:

*"In the assessment of risks, Members shall take into account available scientific evidence; relevant processes and production methods; relevant inspection, sampling and testing methods; prevalence of specific diseases or pests; existence of pest- or disease-free areas; relevant ecological and environmental conditions; and quarantine or other treatment." (Article 5.2)*

and that:

*"In assessing the risk to animal or plant life or health and determining the measure to be applied for achieving the appropriate level of sanitary or phytosanitary protection from such risk, Members shall take into account as relevant economic factors: the potential damage in terms of loss of production or sales in the event of the entry, establishment or spread of a pest or disease; the costs of control or eradication in the territory of the importing Member; and the relative cost-effectiveness of alternative approaches to limiting risks." (Article 5.3)*

## **Questions**

### **Oilseed rape MS1 x RF1 (notification C/UK/94/M1/1)**

*Safeguard measure of France*

**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.3-11; 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?

### **Dr. Nutti**

779. EC161 At. 07–BEC considers that the knowledge which has been acquired makes it possible to contemplate cultivating genetically modified, herbicide-tolerant beet, subject to code of practice being applied and biovigilance measures being instituted. It requires large scale experiments with GM herbicide tolerant oilseed rape, in order to validate methods of managing crops of these varieties. Finally, BEC recommends that overall consideration be given to dealing in detail with the question of introducing new weed control practices which are based on the large scale use of total herbicides. EC161–At. 07 – Summary of BEC, states that it is possible to contemplate cultivating genetically

modified, herbicide-tolerant beet, subject to code of practice being applied and biovigilance measures being instituted.

EC161–At. 08 – Detailed opinion of BEC

780. Based on 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) submitted by application (EC-102/At.1-20) and further information provided by Monsanto/Novartis (EC-102/At.22, 26 and 27-30), my understanding is that there was enough scientific evidence available to France in November 1998 and July 2001, information sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, from the growing for seed production of oilseed rape MS1 x RF1. Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by UK and SCP were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

781. It is important to point out that either the lead CA (UK) and the SCP concluded that there is no evidence to indicate that the placing on the market of the oilseed rape MS1 x RF1, with the purpose to be used as any other oilseed rape is likely to cause adverse effects on human health and on the environment.

**Dr. Andow**

782. France provided reasons for its safeguard measure, beginning in 1998 with EC-161/At.2trans. Here France indicates that the spread of herbicide resistance and the concomitant loss of efficacy of herbicides with small environmental impact are their concerns at the time of implementing the safeguard measure. Specifically France says (in translation)

- Controlling adventitious flora and oilseed rape regrowth in plots in the event of crop rotation and in peripheral areas, would be more complex. Consequently, systematic recourse to molecules with less favourable ecotoxicological profiles may be a necessity;
- the use of molecules with a favourable ecotoxicological profile may be lost because of their proven loss of effectiveness, whereas these molecules are especially used for weeding between crops, at field borders and in various environments such as edges of railway tracks or roads.

The possible developments described above would be worsened by:

- The possibility of an accumulation of non-selective herbicide tolerance genes in double-resistant single lines;
- systematic introduction of these genes in all cultivated species.

783. France also adds that "There is also the question of the impact on agriculture of a possible dependency vis-à-vis only two types of genes and active ingredients."

784. At the center of this is that France believes that the probability of escape of the HT transgene as oilseed rape volunteers or into wild species is not accurately assessed, and that the efficacy and implementation of risk management measures to eliminate HT volunteers and wild species are not accurately evaluated. On the probability of escape, France is concerned because the scientific evidence available at the time comes from a few small-scale dispersal experiments. France believes that when GMHT oilseed rape is commercialized, it will occur at a large spatial scale. Dispersal processes at large spatial scales may exhibit different characteristics than those at small spatial scales. This is contrary to the Opinion of the SCP,<sup>114</sup> although the SCP does not provide a scientific argument against France's concern about spatial scale. France is also concerned that specifying and implementing the good agricultural practices that would eliminate HT volunteers and wild species will be considerably more difficult than merely allowing present agricultural practices to continue in the expectation that present practices will eliminate these undesired plants. This directly contradicts the SCP's opinion,<sup>115</sup> which seems to imply that normal agricultural practices will be sufficient.

785. In the study of dispersal in ecology, during the 1980s and 1990s it became increasingly apparent that measures of dispersal rates and dispersal kernels (dispersal probability as a function of distance) were related to the spatial scale of the measurement. Estimated dispersal rates are smaller when the spatial scale of measurement is smaller. This is probably a general result of the fact the most biological dispersal kernels are highly leptokurtic. This scale-dependence has also been observed for pollen dispersal from oilseed rape. One study from 1995 suggested this<sup>116</sup>, and was later confirmed in a 1999<sup>117</sup> and 2002 publication in *Science*<sup>118</sup> on large-scale pollen dispersal. Thus, the scientific evidence in 1998 on dispersal of oilseed rape pollen was not sufficient to complete an accurate assessment of dispersal probability. Sufficient data appeared by the end of 2002.

786. The scientific basis for believing that normal "good" agricultural practices would be sufficient to eliminate HT volunteers and weedy relatives was not clearly articulated prior to France's 1998 decision. It is likely that the scientific reasoning for believing that normal "good" agricultural practices would be sufficient and easy to implement was as follows. Presently (meaning in 1998)

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<sup>114</sup> CDA-69 SCP Opinion, 18 May 1999 on France's invocation of Article 16 on MS1 x RF1.

<sup>115</sup> CDA-69 SCP Opinion, 18 May 1999 on France's invocation of Article 16 on MS1 x RF1. The SCP states "The available evidence from the scale of release at that time [19 May 1998] suggested that volunteers can be controlled by agronomic practice (cultivation and the use of an alternative broad spectrum herbicide) provided that adequate monitoring procedures are in place to identify spillage, dispersal and any subsequent volunteers." The reference to 19 May 1998 is to CDA-35A SCP Opinion, 19 May 1998. This Opinion is on MS8 x RF3, not MS1 x RF1. The SCP considers that resistance can be managed by a cohesive code of practice supported by a strong education program. Specifically they recommend

"2. The Committee was also of the opinion that the potential transfer of the herbicide resistance gene to wild Brassica relatives is a new issue in Europe in view of the limited scale of release to date. The Committee has examined the available evidence from monitoring and research programmes to date. After evaluating all the information available to the Committee, it was concluded that herbicide-tolerant volunteers that may appear would be canola plants and not wild Brassica relatives. Such herbicide-tolerant volunteers could be controlled in subsequent crops by conventional agricultural methods. The Committee recommends that the introduction of herbicide-tolerant crops should be accompanied by:

i) an agreed code of practice for the particular modified crop involving the active participation of the notifier to promote best practice by farmers.

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."

<sup>116</sup> Timmons et al. 1995 *Euphytica* 85: 417-423.

<sup>117</sup> Thompson et al. 1999 in EC-162/At.13.

<sup>118</sup> Pollen-Mediated Movement of Herbicide Resistance Between Commercial Canola Fields Mary A. Rieger, Michael Lamond, Christopher Preston, Stephen B. Powles, and Richard T. Roush *Science* 28 June 2002; 296: 2386-2388.

oilseed rape volunteers are normally not significant weeds, and in the rare case when they become weeds, they can be easily controlled using the proper herbicide. Weedy Brassica species are important weeds, but these can also be controlled using appropriate agricultural methods. Accepting that this argument is true for the purposes of this paragraph, what remains uncertain is whether the level of weed control that is considered sufficient for normal agricultural practice is sufficient for eliminating HT volunteers and wild species to the level desired by France.

787. Alternatively, the argument might not be true. There may be years when certain fields of GMHT oilseed rape are not harvested at all and the entire crop goes to seed (since this happens occasionally for non-transgenic oilseed rape). The subsequent Brassica weed problem may require exceptional agricultural practices to bring the field under control. Such possibilities were not considered by the SCP, and France may be concerned about such scenarios.

788. Finally, France may be convinced (rightly, I think) that agricultural management of HT volunteers and wild species will not be as simple as the SCP believes. Hence the scientific evidence in 1998 on the agricultural practices necessary to eliminate HT volunteers and wild species was not sufficient to complete an accurate assessment of risk management practices. Based on the material in EC-161, I could not determine when sufficient information was first available. In 2001 the French Biomolecular Engineering Committee (BEC) called for additional research in this area, but by the middle of 2003, the BEC believed that it had sufficient information to complete an assessment.<sup>119</sup>

789. France also could have argued in 1998 that there was insufficient data to identify all of the possible environmental hazards of GMHT oilseed rape. France could have argued that the molecular characterization was insufficient to identify all transgene products. It is not clear to me if MS1 x RF1 hybrids were ever subject to the molecular analyses performed for the GM crops evaluated under Directive 2001/18/EC. France could have argued that the risk of evolution of resistance in weeds not related to the Brassicaceae had been incompletely assessed and management of these risks required additional scientific data. These concerns were widely appreciated in the early 1990s, but action to address them in the EU for GMHT crops did not take place until sometime after 1998. It is not clear to me that adequate information to address this issue exists even as of this date in 2005. France could have argued that the data on non-target effects was insufficient. This claim would have been borne out first by the suggestion in 2000 that GMHT oilseed rape could adversely affect skylarks in the UK followed by the FSE trials in 2003 that suggested some possible adverse effects. Sufficient data were available to assess these risks by October, 2003. Finally, recently France appears to argue that contamination of conventional oilseed rape varieties is an important concern (related to the "coexistence" issue). While this risk seems possible to assess now, it is not clear that the scientific information for justifying the risk management practices is available (this is because I am uncertain about the details of the information available about good agricultural practices noted in paragraph 788).

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<sup>119</sup> EC-161/At.3trans.

**Dr. Snow**

BACKGROUND FOR MY ANSWER

To explain my answer to this question, I include relevant statements from France, the European Commission, and the SCP opinions, along with a few comments below.

**1. France**

France stated (EC-161/At.3 translation): "*These orders banning the placing on the market of genetically modified oilseed rape are based on the risk of contamination of crops of conventional oilseed rape by transgenic oilseed rape. In an opinion given in this regard on 16 February 2001, the Commission du Génie Biomoléculaire (Biomolecular Engineering Committee) concluded that experimentation should be pursued in order to supplement existing scientific knowledge and validate methods for managing the cultivation of genetically modified oilseed rape.*"

Also, "*A further referral to the Biomolecular Engineering Committee by the French authorities on 26 June 2003 aimed to take stock of existing knowledge on the gene flow in oilseed rape and of the identification of risks connected with large-scale cultivation of genetically modified herbicide-tolerant oilseed rape varieties.*"

In addition, in EC-161/At. 4 translation (see excerpts under Question 60), France listed environmental concerns and specific scientific investigations that were needed to address these concerns.

**2. Early European Commission Decision on glufosinate-tolerant oilseed rape (Feb. 6, 1996)**

This favorable decision to place seeds of MS1BnxRF1Bn on the market states that:

*"the survival and spread of this herbicide-tolerant swede-rape was evaluated, as well as the transfer of the herbicide tolerance gene or the other modified genes to compatible species; whereas it was concluded that the risk of establishment was low and that any spread or transfer of the herbicide tolerance gene could be controlled by using existing management strategies."*

As I discuss elsewhere, the assumption that "*the risk of establishment is low*" is not supported by scientific evidence, including evidence that became available during 1998-2003.

**3. Opinions of the Scientific Committee on Plants**

These opinions are nearly identical to others dealing with glufosinate-tolerant oilseed rape. They consider that gene transfer and persistence in wild *Brassica rapa* is unlikely, that potential problems with volunteers can be managed, and that any problems with the spread of herbicide-resistant weeds are agronomic problems rather than environmental ones. The SCP requested that a code of practice and monitoring plans be established to determine whether problems emerge with herbicide-tolerant volunteers and wild relatives.

Opinion of the Scientific Committee on Plants regarding the glufosinate tolerant, hybrid rape derived from genetically modified parental lines (MS8 x RF3) notified by Plant Genetic Systems (notification C/B/96/01) (Submitted by the Scientific Committee on Plants, 19 May 1998)

Excerpts – *"The risk assessment assumes that transfer will occur at a low level. The relevant question is whether this can be contained by risk management and whether it is an environmental or agronomic problem."*

*"Available evidence from the scale of release to date suggests that volunteers can be controlled by agronomic practice (cultivation and the use of an alternative broad spectrum herbicide) provided that adequate monitoring procedures are in place to identify spillage, dispersal and any subsequent volunteers. Normal management methods for wild Brassicae including cultivation, rotation and alternative herbicide should be maintained."*

*"Modified rape is no more invasive than unmodified plants and can be controlled by the combination of cultivation and the use of alternative non-selective herbicides. Potential transgenic exchange is unlikely to lead to establishment as a result of reduced viability of any hybrid plants and competition."*

Opinion of the Scientific Committee on Plants, adopted on 18 May 1999, on the Invocation by France of Article 16 ('safeguard' clause) of Council Directive 90/220/EEC with respect to a genetically modified oilseed rape notification C/UK/94/M1/1 (Plant Genetic Systems N.V.)

Excerpts – *"In its risk assessment the Committee assumed that any such transfer will occur at a low level. It therefore considered the more relevant question, whether this can be contained by risk management and whether it is an environmental or agronomic problem."*

*"The information provided by the French Competent Authority was taken into consideration by the Committee in formulating its original Opinion in 1998 and is not considered to change the environmental assessment and advice to the Commission."*

Answer to Question 59:

790. Given that,

- (i) 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;
- (ii) 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
- (iii) 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;"

791. 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.

792. SUB-QUESTION TO 59. – If so, what scientific evidence do you believe was insufficient?

793. My conclusion is that France needed more information about the rate at which the transgene for glufosinate tolerance would spread to volunteer plants (including the seed bank) and related weeds. France wanted this information in order to determine whether the types of problems that Canada was experiencing (see above) could be managed in the context of the France's agricultural system and, if so, what the cost would be to farmers.

794. By 2003, more scientific knowledge was available in the form of peer-reviewed papers and reports of farmers' experiences in Canada, but new information was still coming to light about the spread and persistence of genes for herbicide tolerance (see Context section above, and References).

### **Dr. Squire**

795. The view expressed by France was that insufficient information was available to provide clear conclusions on several aspects of cross-pollination and persistence of GMHT oilseed rape. The EC Scientific Committee on Plants had considered that the risks arising from outcrossing to volunteer, feral and wild relatives were definite, but small because any progeny could be controlled later in the rotation. France appeared to take a stronger line, indicating the risks were larger or more uncertain. 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. In November 1998 and July 2001, there was knowledge about the mechanisms involved in cross-pollination and persistence, but one of France's main arguments was based on the up-scaling effect – the uncertainty of what might occur if GMHT crops were grown widely in the country (Notes, paragraphs 11, 12). 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.

796. (a) The documentation is also correct when it states that relevant new experimental information was being placed before the public (see example references below). The new information was providing greater knowledge of cross-pollination in oilseed rape, particularly over distances of several kilometres. This new information would not have provided qualitatively different knowledge (it was already known that oilseed rape could cross-pollinate at distance) but would provide better quantitative estimates of the pollination frequencies and the subsequent survival of GM hybrids among volunteer populations. Even by the end of 2003, there was still uncertainty as to what the field-to-field cross pollination might be if GMHT crops came to be grown in many fields throughout a landscape in Europe. Data in Europe was confined to knowledge of crossing from a relatively small source (one or a few fields of donor oilseed rape) to a large potential sink (many fields on recipient oilseed rape). Work is still in progress, not least as one of the main aims of the SIGMEA project (Notes, paragraph 14), to estimate spread and cross-pollination on a regional scale in Europe.<sup>120</sup>

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<sup>120</sup> Rieger MA, Lamond M, Preston C, Powles SB, Roush R. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296, 2386-2388.

Ramsay G, Thompson CE, Squire GR. 2003. Quantifying landscape-scale gene flow in oilseed rape. Final report on Project RG 0216, 48 pp. Defra: London.

**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?**

**Dr. Nutti**

797. The reasons given by France were mainly related to the code of practice being applied and biovigilance measures being instituted. These are environmental issues that I cannot answer but, again, I would like to point out that there was no scientific evidence regarding to food and feed safety assessment, for animal and human consumption, on which France could be based for asking the moratorium.

**Dr. Andow**

798. With reference to the SPS Agreement, France has evaluated relevant risks to plant health, and expressed some of these risks in terms of their consequent potential effect on human health. Specifically, France has taken into account available scientific evidence (Article 5.2).

799. It is not clear, however, that France conducted the risk assessment according to the phytosanitary measures which might be applied. France did not present their safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that France conducted the risk assessment consistent with Annex A, paragraph 4.

800. France did take into account relevant economic factors (Article 5.3), however France did not explicitly compare the relative cost-effectiveness of alternative approaches to limiting risks.

801. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because France argues that there is a plant pest risk.

802. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

803. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of France contradict this guidance. My reading of the materials before the Panel is that France did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1 (level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

804. The science, documentation and reasoning of France are consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Specifically, Annex III, Section 8(f) 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."

**Dr. Snow**

805. With reference to the SPS definition of a risk assessment, in EC-161/At. 4, France stated: *"The main undesirable effects expected from the marketing of genetically modified herbicide tolerant oilseed rapes are therefore agronomic and commercial."*

806. 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. My reasons are explained below.

807. Briefly, France was concerned about the spread of herbicide-tolerant volunteers and related weeds, such as Brassica rapa. This concern was mentioned repeatedly by the EC Scientific Committee on Plants, although the SCP felt that the risk could be managed after commercialization of the product. The French Biomolecular Engineering Committee felt that this should be studied before rather than after de-regulation. This is understandable if one assumes that the spread of herbicide tolerance genes is harmful to the environment and to farmers (see above), because after the crop has been released it could be very difficult to eradicate unwanted transgenes from volunteer, weedy, or cultivated populations.

808. Based on excerpts from the documentation from France and the USA explanation of risk assessments, both of which are included below, the requested research can be viewed as part of a standard risk assessment. The requested research focuses on the "exposure" term reflecting how widely a given harm will occur (as in risk = exposure x harm).

809. The First Written Submission of the USA, point 103, explains that risk assessments should *"(2) evaluate the likelihood of entry, establishment or spread of these diseases [or pests], as well as the associated potential biological and economic consequences."* Detailed empirical studies about the spread of herbicide-tolerance genes by pollen and inter-specific hybrids are useful for estimating 1) how common these genes could become in volunteer and weed populations, 2) whether this problem can be mitigated, and 3) what type of monitoring should be considered after commercial release has occurred.

810. In EC-161/At. 4 translation, France stated:

*"Oilseed rape is widely grown in France, covering 1.1 million hectares in 2003, nearly 3 per cent more than in 2002. It ranks after wheat (4.6 million hectares) and maize (1.8 million hectares). ..."*

*"The main undesirable effects expected from the marketing of genetically modified herbicide tolerant oilseed rapes are therefore agronomic and commercial. It would appear that dispersal, at a rate to be specified, of the herbicide tolerance genes into the environment towards adventitious flora is likely. Furthermore, dispersal into the environment through intraspecific hybridization is inevitable, particularly on the borders of fields and along lanes and roads. Oilseed rape is a highly competitive species and has the capacity to generate regrowth for several years in the following rotation crops ..."*

*"Accordingly, in the context of a referral from the Ministry of Agriculture on 6 July 2000, the Biomolecular Engineering Committee issued an opinion on 16 February 2001 (attached), in which it concluded that experimentation should be pursued so as to supplement existing scientific knowledge and validate methods for managing the*

*cultivation of genetically modified oilseed rape. It found in particular that additional knowledge was needed on:*

- *The effects that would be produced by sources of pollen larger in size than those which had hitherto generated information on the dispersal of oilseed rape pollen;*
- *pollen dispersal over a long distance, and particularly the role of insect transfer;*
- *better characterization of particular interspecific hybrids (amphidiploids), in the interests of a better understanding of the species' dynamics;*
- *the importance of the impact of islands of persistent oilseed rape on the pollination of neighbouring oilseed rape fields, including on those that would result from the escape of seeds in the transport phases, and the dynamics of such islands (persistence, extension, ... )."*

*"A further referral by the Ministry of Agriculture to the Biomolecular Engineering Committee, on 26 June 2003, aimed to take stock of acquired knowledge regarding gene flow in oilseed rape and of the identification of risks related to large scale cultivation of genetically modified herbicide tolerant oilseed rape varieties."*

811. Regarding Question 60 (a), I did not find an ISPM statement about situations that would trigger "safeguard measures" while additional scientific studies are carried out.

**Dr. Squire**

812. As indicated earlier, the requirements in the international guidelines are quite stringent. 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'.

**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?**

**Dr. Nutti**

813. The reasons given by France were mainly related to the code of practice being applied and biovigilance measures being instituted. These are environmental issues that I cannot answer, but again I would like to point out that there were no scientific evidence regarding to food and feed safety assessment, for animal and human consumption, on which France could be based for asking the moratorium.

**Dr. Andow**

814. The adoption of a temporary prohibition can be justified on the basis of the scientific evidence and other information submitted by France.

815. 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 78.

816. 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.

**Dr. Snow**

817. 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.

818. 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.

**Dr. Squire**

819. The risk management options were and are similar to those indicated generally for oilseed rape at Responses 6d and 101. The risk of spread and cross-pollination can be reduced by such measures but not eliminated.

**Topas 19/2 (notification C/UK/95/M5/1)**

**Question 62: Given that oilseed rape Topas 19/2 was only approved for import of seeds for processing, what is the relevance of the findings of the UK farm scale evaluation study (EC-38) in terms of the assessment of potential risks from Topas 19/2?**

**Dr. Nutti**

820. In the study presented in EC-38, the researchers monitored plants and animals in the field and around ploughed edges of the fields called field margins. This was done before, during and after the crops were grown and harvested. It was an environmental study, in order to give information for the environmental assessment. In my opinion, this study has no relationship with the safety assessment of Topas 19/2, as the permission for this product was asked for "import of seeds for processing", and not for cultivation in Europe. My conclusion is that this study has no relevance for the assessment of potential risks of a product that will be only processed and not cultivated in Europe.

**Dr. Andow**

821. The findings of the UK FSE study (EC-38) are not relevant to the risk assessment for import and processing of Topas 19/2. Their only possible relevance would be in the hazard identification step of risk assessment. However, it is unclear how Topas 19/2 could reach such widespread levels of actual (accidental) cultivation to cause such a hazard. It would require escape during importation and/or processing and several years of multiplication at levels similar to the multiplication during oilseed rape seed production and the growing of these large quantities on the landscape. Such large quantities would necessitate actual cultivation, presumably without the knowledge that the seed is derived from Topas 19/2. Even if such a scenario were possible, there would many, many possible ways to manage this risk.

**Dr. Snow**

822. Because the seeds of Topas 19/2 were not intended for cultivation, the UK farm scale evaluation study bears little or no relevance to this decision. I assume that any seed spillage that might occur when the seeds are delivered could be managed, and that it is extremely unlikely that the seeds would be cultivated inadvertently. (It appears that Topas 19/2 was approved for cultivation in the UK in 1996 (EC-162 At. 002-003), but I assume that this earlier decision is not applicable here.)

823. In EC-162 At. 007, Greece states that "it is certain that the seeds will escape into the environment and will give viable plants" in Greece. Even if this is the case, I do not think the UK farm scale evaluations are relevant to the possibility that feral populations of herbicide-tolerant oilseed rape would become established. The farm scale evaluations would only be relevant if the product were cultivated widely.

**Dr. Squire**

824. Topas 19/2 and the Farm scale evaluations (FSE).

825. The FSE compared the effects of GMHT and conventional weed management. Its results should have no bearing on the risk associated with the importation of seeds for processing. Even if these seeds were spilled and became part of the wayside feral population or volunteer population in a field, they would have no effect unless the management of the field changed away from current management in a way comparable to the differences between GMHT and conventional cropping in the FSE.

*Safeguard measure of France*

**Question 63: Given the information before the Panel, including the evaluations undertaken by the UK (EC-162/At.1 to 3), and the SCP in February 1998 (CDA-63), May 1999 (CDA-65 and CDA-73), and the European Commission in its Decision of April 1998 (CDA-61), as well as the information submitted by France with respect to its safeguard measure (EC-162/At/5, EC-161/At.3-11, CDA-64, CDA-66, CDA-67), 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 import and processing of oilseed rape Topas 19/2? 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 import and processing of oilseed rape Topas 19/2? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

826. Answer 63: In EC-162/At.003, the lead CA (UK), having assessed the potential risks to human health and environment arising from the genetic modification, proposes to consent to the placing on the market of the product for specific uses, such as import of Gm seed for processing of food, animal feed and industrial uses of non-living processed products arising from the product; growing and multiplication of seed for breeding material and for sale; field cropping for grain production for feed, food and industrial uses of non-living processed products arising from the product and had specified the conditions and labelling for this uses.

827. In CDA-63, it can be observed that in February 1998 the SCP made it clear that the safety evaluation was done for importation of seeds only, for placing on the market for food and feed purposes. The SCP concludes that there was no significant risk to human and livestock following ingestion of CM seeds. They considered that the GM product and products thereof were substantially equivalent to the traditional counterpart. SCP concluded that there was no evidence to indicate that the placing on the market of the oilseed rape Topas 19/2, with the purpose to be imported for processing and placing on the market for food and feed uses, is likely to cause adverse effects on human health and on the environment.

828. On CDA-65, it can be observed that on May 1999 the SCP was consulted by the Commission on the dossier for Topas 19/2 and the prohibition from the French CA, with concerns on the environmental impact of genetic escape through volunteers and hybrids, and the agricultural consequence of the spread of herbicide tolerance in both cultivated and non-cropped habitats. In its comments, the SCP emphasizes that the application was for the importation of oilseed rape into Europe for processing and not for cultivation and production within member states. The SCP also explains that the potential for the loss of material during transport and the possible establishment of feral plants in uncultivated habitats was considered in the risk assessments carried out by SCP in forming its opinion of February 1998. They concluded that the risk of genetic escape from this market consent was considered by the SCP to be small and the current information submitted by French authorities did not change that assessment.

829. Based on the information presented by the notifiers and by the evaluation of SCP, my understanding is that there is NO 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 import and processing of oilseed rape Topas 19/2.

830. Answer 63a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Dr. Andow**

831. This is a notification for importation and processing, not for cultivation (despite EC-162/At.1-3 stating that the UK evaluates it for cultivation).

832. France relies on the same rationale here as used for MS1 x RF1 (Questions 59-61), which I have reviewed previously in paragraphs 782-783 and will not repeat here. The key difference is that

MS1 x RF1 was for cultivation and seed production as well as importation and processing. There are also minor, formal differences in the Opinion of the SCP that have no consequence for this discussion.<sup>121</sup>

833. The argument used by France that was based on spatial scale does not hold for this case. With importation and processing, it is highly unlikely that large-scale production of GMHT oilseed rape is possible. Thus, the data on small-scale dispersal is probably sufficient to complete a risk assessment even in 1998.

834. Moreover, the hazard of widespread HT resistance in oilseed rape or related wild species does not hold for this case. This hazard is also scale-dependent, and with the small-scale releases and poor survival expected in spillage and other accidental releases, it is not feasible that this hazard will occur. Even if it were possible (see paragraph 821), there would many, many other possible ways to manage this risk short of a prohibition. Thus, the rationale stated by France does not argue that a risk exists that requires management.

835. France also could have argued in 1998 that there was insufficient data to identify all of the possible environmental hazards of GMHT oilseed rape. (1) France could have argued that the molecular characterization was insufficient to identify all transgene products. France could have concluded that there may be risks stemming from these unidentified transgene products (if they exist) that would require a safeguard measure. It is not clear to me if Topas 19/2 was ever subject to the molecular analyses performed for the GM crops evaluated under Directive 2001/18/EC. (2) Recently France appears to argue that contamination of conventional oilseed rape varieties is an important hazard (related to the "coexistence" issue). This is a hazard that could occur from the small-scale releases expected by accidental spillage. The risk associated with this hazard is probably possible to assess now, but I do not have access to the scientific information on the risk management practices available to me to make a more certain statement.

836. Such possible hazards would have allowed France to argue that there is a risk, and that the risk management measures proposed by the SCP were not supported sufficiently by science.<sup>122</sup> 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. Based on the material in EC-161 and EC-162, I could not determine when sufficient information was first available. The information needed would be substantially less than for MS1 x RF1.

837. Given the information before the Panel, France could have argued in November 1998 that there was insufficient evidence to complete a risk assessment. However, I believe that had France collected the necessary data to complete this risk assessment promptly after implementing their temporary prohibition that they could have had sufficient data to make a decision in 2001.

**Dr. Snow**

838. In summary, I did not find any convincing arguments for why further scientific research would be needed to investigate environmental effects of a product that is only intended for import and processing and not for cultivation.

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<sup>121</sup> CDA-63, CDA-65, CDA-73.

<sup>122</sup> This is discussed in more detail in my response to question 59, paragraph 786.

839. See ANSWER 62 for explanation. Because the seeds of Topas 19/2 were not intended for cultivation, the need for environmental assessments appears to be very low to non-existent. I assume that any seed spillage that might occur when the seeds are delivered could be managed and that it is extremely unlikely that the seeds would be cultivated inadvertently. The only conceivable risk that I can imagine is that feral volunteer populations would cross-pollinate with weedy Brassica rapa, giving this weed the trait of resistance to glufosinate, and the weeds would become more abundant because farmers would repeatedly spray these populations with glufosinate (killing off other weeds and favoring the proliferation of the herbicide-resistant weeds). This scenario seems extremely unlikely for the case of GM seeds that are imported for processing and not cultivation.

840. I do not understand why France states "*These orders banning the placing on the market of genetically modified oilseed rape are based on the risk of contamination of crops of conventional oilseed rape by transgenic oilseed rape*" with regard to Topas 19/2 (EC-161 At. 003 translation).

841. ANSWER 63 (a): My conclusion is that I did not find any convincing arguments for why further scientific research would be needed to investigate environmental effects of a product that is only intended for import and processing and not for cultivation. See ANSWERS 62 and 63.

**Question 64: 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 import and processing of oilseed rape Topas 19/2?**

- (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?**

**Dr. Nutti**

842. Environmental issues are not my field, so I can not judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by UK and SCP were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

**Dr. Andow**

843. With reference to the SPS Agreement, France did not identify a relevant risk to plant health. However, it would have been possible to identify a relevant risk to plant health (paragraph 789). Specifically, France did not fully take into account available scientific evidence (Article 5.2). As a consequence, the argument was undercut by a faulty analysis and France missed opportunities to make a stronger scientific argument.

844. Under the assumption that France had identified a relevant risk to plant health, it is not clear that France conducted the risk assessment according to the phytosanitary measures which might be applied. France did not present the safeguard measure as a possible phytosanitary measure. France did not conduct the risk assessment according to this proposed safeguard measure. Thus it is not clear that France conducted the risk assessment consistent with Annex A, paragraph 4.

845. France did take into account relevant economic factors (Article 5.3), however France did not explicitly compare the relative cost-effectiveness of alternative approaches to limiting risks.

846. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case. While France did not identify a potential pest risk, it was possible to identify a potential pest risk under which ISPM 11 could be considered. Thus, ISPM 11 could be invoked.

847. For those risks within the scope of ISPM 11 the risk assessment process would be consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

848. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of France contradict this guidance. My reading of the materials before the Panel is that France did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1 (level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

849. The science, documentation and reasoning of France are consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Specifically, Annex III, Section 8(f) 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."

**Dr. Snow**

850. ANSWERS 64 and 64 (a): My conclusion is that I did not find any convincing arguments for why further scientific research would be needed to investigate environmental effects of a product that is only intended for import and processing and not for cultivation, so the question about international guidelines seems irrelevant. See ANSWER 63.

**Question 65: Does the scientific evidence and other information submitted by France support the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2? 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?**

**Dr. Nutti**

851. As far as my knowledge goes, the scientific evidence submitted by France does not support a temporary prohibition on the import and processing of oilseed rape Topas 19/2.

**Dr. Andow**

852. The scientific evidence and other information submitted by France does not support the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2. However, scientific evidence and other information before the Panel could be used to support the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2. Even on these grounds, a temporary prohibition probably could not be justified past 2001.

853. Several other risk management options could have been justified in November 1998. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. In 1998 mitigation and tolerance strategies were probably inappropriate. Another risk avoidance strategy would have been to request additional molecular characterization data and allow limited importation with attendant monitoring so that the practical aspects of controlling escapes from spillage could be experienced.

854. These options were available in 2001 and remain today. In addition risk mitigation strategies may also be possible to control HT volunteers and wild species.

**Dr. Snow**

855. In summary, I did not find any convincing arguments for why further scientific research would be needed to investigate environmental effects of a product that is only intended for import and processing and not for cultivation, so the question about risk management options seems irrelevant. See ANSWER 63.

*Safeguard measure of Greece*

**Question 66: Given the information before the Panel, including the evaluations undertaken by the UK (EC-162/At.1 to 3), and the SCP in February 1998 (CDA-63), May 1999 (CDA-65 and CDA-73), and the European Commission in its Decision of April 1998 (CDA-61), as well as the information submitted by Greece with respect to its safeguard measure (EC-162/At. 4, CDA-72), is there any reason to believe that the scientific evidence available to Greece in September 1998 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 import and processing of oilseed rape Topas 19/2? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in September 1998, was there sufficient evidence available to Greece 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 import and processing of oilseed rape Topas 19/2 (EC-162/At.6 to 13)? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

856. Answer 66: On CDA-73, the SCP answers the points raised by the Greek CA, and does not change their previous risk assessment. The SCP explains that the potential for the loss of material during transport and the possible establishment of feral plants in uncultivated habitats was considered in the risk assessments carried out by SCP in forming its opinion of February 1998. The SCP points out that in the absence of the use of glufosinate ammonium to apply selective pressure, modified rape is not more invasive than unmodified rape and oilseed rape is not grown commercially in Greece. They concluded that the risk of genetic escape from this market consent was considered by the SCP to be small and the current information submitted by Greek authorities does not change that assessment.

857. Based on the information presented by notifiers and by the evaluation of SCP, my understanding is there is NO reason to believe that the scientific evidence available to Greece on September 1998 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 import and processing of oilseed rape Topas 19/2.

858. Answer 66a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Dr. Andow**

859. This should be considered a continuation of my responses to Questions 62-65. In EC-162/At.7 Greece identifies three risks to human, plant or animal health for which there is insufficient evidence to undertake an appropriate assessment. These are: 1. harm of wildlife and the environment;<sup>123</sup> 2. human health risk from outcrossing to wild Brassica species (e.g., Brassica cretica) in Greece that are used as food; 3. increased difficulty in managing farmland because GMHT oilseed rape overwinters better in Greece, should spread faster and is hard to eliminate once established as a weed.

860. In paragraph 821, I suggested that the first of these is not relevant to Topas 19/2, which is a notification for import and processing. The SCP Opinion states that there is no evidence that the known gene products of the Topas 19/2 transformation are a human health risk,<sup>124</sup> and Greece does not address this explicitly. Thus, there is little merit in the second risk. There is some merit in the third risk. On this point there is a clear difference in the Opinion of the SCP, which considers this remote, and Greece, which does not. Oilseed rape survives better in Greece than in France, so while this risk is probably very small in France, it is likely to be larger in Greece. It is probably fair to conclude that it is not certain how much higher this may be. However, if this risk were substantially larger than believed by the SCP, there would many other possible ways to manage this risk short of a prohibition.

861. Greece also could have argued in 1998 that there was insufficient data to identify all of the possible environmental hazards of GMHT oilseed rape. (1) Greece could have argued that the molecular characterization was insufficient to identify all transgene products. Greece could have concluded that there may be risks stemming from these unidentified transgene products (if they exist) that would require a safeguard measure. It is not clear to me if Topas 19/2 was ever subject to the molecular analyses performed for the GM crops evaluated under Directive 2001/18/EC. (2) Recently Greece appears to argue that contamination of conventional oilseed rape varieties is an important hazard (related to the "coexistence" issue). This is a hazard that could occur from the small-scale releases expected by accidental spillage. The risk associated with this hazard is probably possible to assess now, but I do not have access to the scientific information on the risk management practices available to me to make a more certain statement.

862. Risk 3 (paragraph 859) and such possible hazards as in paragraph 861 would allow Greece to argue that there is a risk, and that the risk management measures proposed by the SCP were not supported sufficiently by science.<sup>125</sup> 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. In addition, Greece may have believed that implementing "good" agricultural practices would be more complicated than anticipated by the SCP. Based on the material in EC-161 and EC-162, I could not determine when sufficient information was first available. The information needed would be substantially less than for MS1 x RF1 (questions 59-61).

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<sup>123</sup> This complaint foreshadows the results from the UK-FSE trials and models of skylark impact

<sup>124</sup> CDA-63, CDA-73.

<sup>125</sup> This is discussed in more detail in my response to question 59, paragraph 786.

863. Given some of the information provided by Greece and other the information before the Panel, Greece could have argued in November 1998 that there was insufficient evidence to complete a risk assessment. However, I believe that had Greece collected the necessary data to complete this risk assessment promptly after implementing their temporary prohibition that they could have had sufficient data to make a decision in 2001.

**Dr. Snow**

864. See ANSWERS 63-65. I did not find any convincing arguments for why further scientific research would be needed to investigate environmental effects of a product that is only intended for import and processing and not for cultivation. The specific arguments provided by Greece seem flawed. I do not understand why Greece assumes that feral populations would become established or why they would cause environmental problems. Even if such populations become established, there is no scientific reason to expect that gene flow could harm the genetic diversity or abundance of wild relatives of oilseed rape in Greece. The arguments that Greece made for additional research appear to be based on the assumption that the product would be cultivated rather than imported only for processing.

**Question 67: 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 Greece evaluate the relevant risks to human, plant or animal health, and the environment from the import and processing of oilseed rape Topas 19/2?**

- (a) **How does the scientific evidence and other documentation submitted by Greece compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

865. Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by UK and SCP was adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

**Dr. Andow**

866. With reference to the SPS Agreement, Greece has identified one relevant risk to plant health.<sup>126</sup> In addition, it is also possible to identify other relevant risks to plant health. Specifically, in some places Greece did not fully take into account available scientific evidence, but in others it did (Article 5.2). In addition Greece missed opportunities to make scientific arguments in its favor.

867. It is not clear that Greece conducted the risk assessment according to the phytosanitary measures which might be applied. Greece did not present the safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to this proposed safeguard measure. Thus it is not clear that Greece conducted the risk assessment consistent with Annex A, paragraph 4.

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<sup>126</sup> Risk 3, paragraph 859.

868. Greece did take into account relevant economic factors (Article 5.3), however Greece did not explicitly compare the relative cost-effectiveness of alternative approaches to limiting risks.

869. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case, because Greece did identify a potential risk. In addition, other potential risks relevant to ISPM 11 could be identified.

870. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

871. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Greece contradict this guidance. My reading of the materials before the Panel is that Greece did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1 (level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

872. 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) 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."

**Dr. Snow**

873. See ANSWER 66.

**Question 68: Does the scientific evidence and other information submitted by Greece support the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2? In light of any potential risks identified by Greece, what other risk management options were available in September 1998? What other risk management options are now available?**

**Dr. Nutti**

874. As far as my knowledge goes, the scientific evidence submitted by Greece does not support a temporary prohibition on the import and processing of oilseed rape Topas 19/2.

**Dr. Andow**

875. Some of the scientific evidence and other information submitted by Greece supports the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2. In addition, scientific evidence and other information before the Panel could be used to support the adoption of a temporary prohibition on the import and processing of oilseed rape Topas 19/2. Even on these grounds, the temporary prohibition probably could not be justified past 2001.

876. Several other risk management options could have been justified in November 1998. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. In 1998 mitigation and tolerance strategies were probably inappropriate. Another risk avoidance strategy would have been to request additional molecular characterization data and allow limited importation with

attendant monitoring so that the practical aspects of controlling escapes from spillage could be experienced.

877. These options were available in 2001 and remain today. In addition risk mitigation strategies may also be possible to control HT volunteers and wild species.

**Dr. Snow**

878. See ANSWER 66.

**Maize Bt-176 (notification C/F/11-03)**

*Safeguard measure of Austria*

**Question 69: Given the information before the Panel, including the evaluations undertaken by France (EC-158/at. 1 to 3); the Scientific Committee for Animal Nutrition in December 1996 (EC-158/At. 4 and 5); the Scientific Committee for Food in December 1996 (US-64) and in March 1997 (US-58); the Scientific Committee for Pesticides in December 1996 (EC-158/At.6\_SCI) and in May 1997 (US-57); the Scientific Committee for Plants (SCP) in September 2000 (US-66); and the European Commission in its Decision of January 1997 (ARG-37), as well as the information submitted by Austria with respect to its safeguard measure (US-52, EC-158/At.11, 12 and 15; EC-144, EC-147), is there any reason to believe that the scientific evidence available to Austria in February 1997 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 importation and use of Maize Bt-176? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in February 1997, was there sufficient evidence available to Austria 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 importation and use of Maize Bt-176 (EC-158/At.30 to 42)? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

879. Answer 69: In EC158-At.04, the Scientific Committee for Animal Nutrition, concludes that the probability of the transfer of a functional bla-gene construct from GM maize to bacteria is virtually zero and if this virtually impossible event would take place, it would have no clinical significance. The conclusion also pointed out that there is no evidence of a risk of causing B-lactam antibiotic resistance in animal bacteria with the use of the GM maize. The SCAN opinion was that there is no evidence indicating that the use in animal feeding of the GM maize will give rise to any adverse effect on animal health.

880. In EC158-At. 05, the Scientific Committee for Food concludes that the GM maize is, except for the inserted traits, substantially equivalent to maize present on the market. Animal feeding studies support its substantial equivalence to the parent plant. No nutritional concerns are associated with the use of this transgenic line; and it is unlikely that the genetic changes introduce any new potential for allergenicity. No human toxicological concerns arise regarding the inserted traits based upon the toxicological and degradation data considered. The conclusion also stated that the possibility that the product would add significantly to the already widespread occurrence of ampicillin resistant bacteria in animals and man is remote and the risk of bacterial transformation is extremely low. The committee recognized the general question of using genes coding for antibiotic resistance for the

development of novel foods and propose to scrutinise future needs and applications of this marker genes. In March 1997 (US-58), the Scientific Committee for Food stated that the Austrian information does not provide new scientific evidence regarding the food use of Ciba-Geigy maize that was not taken into account by the Committee at the time its opinion was delivered. Thus, the Austrian information does not cause the SCF to consider that the GM maize constitutes a risk to human health.

881. In EC158-At. 06\_SCI, the Scientific Committee for Pesticides pointed out the glufosinate ammonium was not authorized for direct application into maize plants, and should not, therefore, be used for post emergence on genetically modified maize without authorization, in accordance with provisions of Directive 91/414/EEC. As far as the Bt event is concerned, the Scientific Committee for Pesticides was of the opinion that the possible development of insect resistance to the Bt toxin arising from the cultivation of maize plants containing Bt-toxin would not be an adverse effect on the environment.

882. In EC158-At. 07, the Austrian CA informs the applicant that the marketing of Ciba Geigy Maize in Austria has been prohibited by an ordinance, which entered in force on 14<sup>th</sup> of February of 1997, and this action has been in accordance with Art. 16 of Directive 90/220/EEC. The lead CA recognizes that expert from different scientific committees have extensively reviewed and discussed problems, concluding the lower risk of the product and all the scientific comments and arguments are valid and well taken, however, from the Austrian point of view, especially new scientific results have questioned the present scientific possibility of conclusive evaluation of the mechanism of gene transfer as well as the development of resistance to Bt toxin.

883. The European Commission, in its Decision of January 1997, has given consent to the placing on the market of the Ciba Geigy Maize and any progeny derived from crosses of this product with any traditionally bred maize.

884. Based on the information presented by the notifiers and by the evaluation of four different scientific committees, my understanding is there is NO reason to believe that the scientific evidence available to Austria in February 1997 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 importation and use of Maize Bt-176.

885. Answer 69a: In my opinion, the scientific evidence presented was sufficient in 1997 and 2003.

**Dr. Andow**

886. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

887. The initial assessment of Event 176 by France provided in EC-158/At.1-3 (10 January 1995, 1 March 1995, 3 March 1995) does not contain the details of the assessment by the French Biomolecular Engineering Committee (BEC).

888. The Opinion of the Scientific Committee on Pesticides (EC-158/At.6\_SCI, 9 December 1996) on Event 176 did not assess the risk of resistance evolution in the target pests because it considered this risk to be an agricultural risk, not an environmental risk. In either event, the Committee felt that resistance management should be fully considered. The Committee also did not assess non-target effects.

889. The Opinion of the SCAN (EC-158/At.4, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on animal health. The Opinion of the SCF (US-64, EC-158/At.5, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on human health.

890. On 23 January 1997, the EC decided to allow the placing on the market of Event 176 (ARG-37).

891. On 13 February 1997, Austria decided to ban the commercialization of Event 176 in Austria (US-52). Austria gave three reasons for this action. 1. A concern that the risks of the bla gene (ampicillin resistance) are greater than that assessed by the SCAN and the SCF (paragraph 889). 2. That the Cry1Ab toxin in Event 176 could have risks to non-target organisms, including those in the soil such as collembola. 3. There is an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin, and resistance management measures should be required (they were not required by the EC).

892. In the Further Opinion of the Scientific Committee on Pesticides (US-57, 12 May 1997), "The Committee concluded that the reasons and information submitted by the Austrian Authorities did not add new relevant evidence to that already considered by the Committee and that none of its conclusions on the risk to the environment were affected by the Austrian arguments."

893. The reasoning in the Further Opinion of the Scientific Committee on Pesticides (US-57, 12 May 1997) is false and did not take into account the scientific information known at the time. The conclusions it reached (paragraph 892) do not follow from the evidence available at the time.

894. (1) On page 2, the Committee stated that it "recognized the complexity of comparing the exposure of a pest to genetically modified plants, where exposure may be prolonged and maintained, and conventionally applied pesticides, with shorter and repeated exposure. The potential for development of resistance could be either accelerated or retarded." (a) In its 9 December 1996 Opinion, the Committee nowhere recognized the complexity of comparing the exposure of a pest to genetically modified plants, where exposure may be prolonged and maintained, and conventionally applied pesticides, with shorter and repeated exposure. It must be granted that this is a new consideration for the SCP that was introduced by Austria. (b) While it is true that theoretically the difference in exposure could result in faster or slower resistance evolution, the SCP is not discussing a theoretical case. There was more than adequate data available in 1995 from the notifier's efficacy trials (and efficacy trials on Event 176 conducted by public sector scientists) and a long history of efficacy trials of Bt insecticide sprays on maize to demonstrate without question that Event 176 would exert a selection pressure on corn borers many times stronger (I would estimate ~1000x) than a typical Bt insecticide spray. The conclusion of the Committee either is not case-specific and therefore inappropriate, or it is case-specific and therefore false.

895. (2) On page 2, the Committee stated "Based on available information, soil exposure from the GMO maize plants will be less than the exposure resulting from a single conventional spray application including the run-off from the plants. Only trace amounts of toxin can be detected in the roots of the maize and furthermore, normal agricultural practice would involve removal of the greater part of the plant at harvest. The plant remains are often shredded and transformed into silage for use as animal feed at a later stage." (a) In its 9 December 1996 Opinion, the Committee nowhere made the assessment that based on available information, soil exposure from the GMO maize plants will be less than the exposure resulting from a single conventional spray application including the run-off from the plants. Nowhere did the Committee note that these issues were addressed satisfactorily in the dossier submitted by the notifier. It must be granted that these considerations are new to the

Committee and that they were introduced by Austria. (b) The conclusion that soil exposure from the Event 176 maize plants will be less than the exposure from a single Bt spray application was debatable even in May 1997. The Committee did not appear to take into account that nearly all of the Cry toxin applied in a Bt spray would be inactivated by sunlight in less than week after spraying. The Committee did not appear to consider that most farmers incorporate maize residue into the soil after harvest. This would incorporate several tons of biomass per hectare, putting Cry toxin out of the sun, where it could persist for some time. The Committee seemed to believe that the maize residue would be ensiled. Silage requires green plant material, and most silage maize is cut while still green, long before it would be harvested for grain. Thus it is not clear that very much residue would be removed for silage. Finally risk assessments should not rely on one study for key conclusions (in this case the Palm et al. study, cited as study 4 in the Opinion). The SCP assessment should have acknowledged that scientific information was scarce at that time, and it would have been better had the SCP maintained a more agnostic position with respect to the risk assessment. Thus, I conclude that the Committee did not consider all available scientific evidence and did not appropriately weigh potentially conflicting information when coming to their conclusion.

896. (3) On page 2-3, the Committee states "The Committee stated in its previous report of 9 December 1996 that resistance management strategies are needed during the years of use of any pesticide, Bt sprays included. The Committee drew attention once again to the need for effective resistance management, including monitoring on agronomic grounds, to prolong the effectiveness of Bt toxin both in conventional sprays and in genetically modified maize. It also felt that the submission of a satisfactory monitoring and resistance management programme should be a requirement for the authorization to use genetically modified maize seeds expressing Bt-toxin." (a) In its 9 December 1996 Opinion, the Committee nowhere made the recommendation that the submission of a satisfactory monitoring and resistance management programme should be a requirement for the authorization to use genetically modified maize seeds expressing Bt-toxin. The acknowledgement that it should be a requirement in this 12 May 1997 Opinion is a major change in the conclusions and recommendations of the Committee. It must be granted that this change was due to Austria's insistence that resistance management measures are required for commercial authorization. (b) The Committee does not address Austria's main point on this issue, which is that resistance risk should be considered an environmental risk. The Committee also seems to be unaware of the position of the US-EPA in 1996, which was that resistance risk is an environmental risk.<sup>127</sup> This is a critical issue because it determined whether resistance risk could be considered under Directive 90/220/EEC. Thus, the Committee did not engage on a critical reason submitted by Austria.

897. (4) It must be granted that the reasons and information submitted by the Austrian Authorities added new relevant evidence that the Scientific Committee on Pesticides had not considered in their previous deliberation, and that that a key conclusion was affected by the Austrian arguments. Moreover, the key conclusions by the Committee in this Opinion on non-target risks are overstated and/or false.

898. I conclude that the scientific evidence available to Austria in February 1997 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to plants and the environment from Event 176. The evidence on risks to non-target organisms, including those in the soil such as collembola was insufficient. Austria could have mustered sufficient evidence to argue that there was an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin, and resistance management measures should be required. There was probably insufficient evidence

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<sup>127</sup> Matten, S.R., et al., The U.S. Environmental Protection Agency's role in pesticide resistance management, in *Molecular Genetics and Evolution of Pesticide Resistance*, T.M. Brown, Ed., American Chemical Society, Washington, D.C., 1996, pp. 243-253.

available to determine what measures should be required. This determination by Austria was in direct contradiction to the EC decision. In this case, Austria probably did not then and does not now need to claim that their safeguard measure is a precautionary one.

899. By 2003, the basis for risk assessment of Bt crops had 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. Annex II of Directive 2001/18/EC is one consequence, but additional regulatory changes have occurred since then. Indeed, knowledge in molecular biology continues to accumulate at remarkably fast rates, and I expect that there will be continued change in regulation in the future.<sup>128</sup> (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.

900. Thus, evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1997 to 2003. This is particularly true in Europe and the United States, and particularly true for the Bt crops.

901. (1) Thus, in 2003, there remains some uncertainty about non-target risks of Event 176, but it could be argued that a risk assessment could be conducted using scientifically justified worst-case assumptions. On the other hand, Austria could reasonably maintain that there is still insufficient information to know which non-target species may be at risk, and therefore it is not possible to conduct an objective risk assessment. The findings in 2003 by Székács and Darvas (EC-158/At.37) – that two protected butterfly species in Hungary, *Inachis io* and *Vanessa atalanta*, might be exposed to Bt corn pollen and suffer higher mortality – certainly suggests that not all of the non-target species at risk to Event 176 have been identified in Europe. (2) As in 1997, Austria could muster sufficient evidence in 2003 to argue that there is an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin in Event 176, and resistance management measures should be required. Moreover, in 2003, Austria should have sufficient evidence to determine what kind of resistance management measures to require. (3) Unlike in 1997, in 2003 Austria could argue that the molecular characterization of Event 176 is insufficient to conduct a risk assessment. (4) In 2004, Austria suggested that an additional gene flow risk that needs assessment is contamination of conventional production. It is possible that the scientific evidence to support risk management measures for this risk is available to conduct an objective risk assessment today.

**Question 70: 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 Austria evaluate the relevant risks to human, plant or animal health, and the environment from the importation and use of Maize Bt-176?**

- (a) **How does the scientific evidence and other documentation submitted by Austria compare with the relevant international guidelines for risk assessment and analysis identified above?**

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<sup>128</sup> Possibilities are suggested in Nap et al. 2004. *Genomics for Biosafety in Plant Biotechnology*. IOS Press, NATO Science Series, Amsterdam.

**Dr. Nutti**

902. Answer 70: Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the Scientific Committee for Animal Nutrition, the Scientific Committee for Food, the Scientific Committee for Pesticides and the Scientific Committee for Plants (SCP) were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

**Dr. Andow**

903. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

904. With reference to the SPS Agreement, Austria has evaluated relevant risks to plant health. Specifically, Austria has taken into account available scientific evidence (Article 5.2).

905. It is not clear, however, that Austria conducted the risk assessment according to the phytosanitary measures which might be applied. Austria did not present their safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that Austria conducted the risk assessment consistent with Annex A, paragraph 4.

906. Austria did take into account relevant economic factors (Article 5.3), however it did not explicitly compare the relative cost-effectiveness of alternative approaches to limiting risks.

907. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because Austria argues that there is a plant pest risk.

908. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

909. It appears that some of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Austria contradict this guidance. My reading of the materials before the Panel is that Austria did not explicitly address this guidance. Specifically I note Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

910. In US-52, Austria made it clear that they are applying a different standard for acceptable risk than reflected in the assessments of the SCAN, SCF, Scientific Committee on Pesticides in the EC decision (ARG-37) itself. In so doing, Austria fulfilled ISPM 11, Section 3.1 (level of acceptable risk should be expressed).

911. ISPM 11, Section 3.4.6 (prohibition) states "If no satisfactory measure to reduce risk to an acceptable level can be found, the final option may be to prohibit importation of the relevant commodities. This should be viewed as a measure of last resort and should be considered in light of the anticipated efficacy, especially in instances where the incentives for illegal import may be significant." With respect to resistance risk, in 1997 Austria had established the existence of an environmental risk, and required the development of measures to manage this risk to acceptable

levels. As the EC was not willing to make such a requirement, Austria had no choice but to intervene with its own safeguard measure. In 1997, however, there was probably insufficient scientific evidence to establish what necessary measures should be taken. Scientifically credible suggestions at that time focused on using refuges (maize that was not Bt maize) ranging from 10-70% of the maize grown by any farmer who chose to grow Bt maize. It is unlikely that Austria could have implemented any of these suggestions in time for the 1997 growing season, so I conclude that Austria had fulfilled ISPM 11, Section 3.4.6, at least for the 1997 growing season.

912. The science, documentation and reasoning of Austria are consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Specifically, Annex III, Section 8(f) 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."

**Question 71: Does the scientific evidence and other information submitted by Austria support the adoption of a temporary prohibition on the importation and use of Maize Bt-176? In light of any potential risks identified by Austria, what other risk management options were available in February 1997? What other risk management options are now available?**

**Dr. Nutti**

913. It is important to point out that the main issues raised by Austria were related to the present scientific possibility of conclusive evaluation of the mechanism of gene transfer as well as the development of resistance to Bt toxin. As far as my knowledge goes, concerning food safety and nutrition, the scientific evidence submitted by Austria does not support a temporary prohibition on the import and use of Maize Bt-176.

914. In EC-144, Austrian Contribution for the WTO-Dispute-Expert-Meeting of the European Commission 14 January 2004 in Brussels, I found that Austria presents also issues related to allergenicity and toxicological risk assessment, but I cannot agree with the points raised, as they are not in accordance with the FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment.

**Dr. Andow**

915. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

916. The adoption of a temporary prohibition can be justified on the basis of the scientific evidence and other information submitted by Austria.

917. Other risk management options could not have been justified in February 1997. However, had Austria worked to develop its own acceptable resistance management measures, perhaps by 1999 other risk management options would have been possible.

918. Today several alternative risk management options are available. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. Tolerance strategies are probably inappropriate. One alternate risk avoidance strategy would be to implement country-specific resistance management measures, to limit planting to a restricted region, and to conduct intensive non-target experiments. This would allow progressive determination of non-target effects.

*Safeguard measure of Germany*

**Question 72:** Given the information before the Panel, including the evaluations undertaken by France (EC-158/at. 1 to 3); the Scientific Committee for Animal Nutrition in December 1996 (EC-158/At. 4 and 5); the Scientific Committee for Food in December 1996 (US-64) and in March 1997 (US-58); the Scientific Committee for Pesticides in December 1996 (EC-158/At.6\_SCI) and in May 1997 (US-57); the SCP in September 2000 (US-66); and the European Commission in its Decision of January 1997 (ARG-37); as well as the information submitted by Germany with respect to its safeguard measure (US-65, EC-158/At.18-29, EC-144), is there any reason to believe that the scientific evidence available to Germany in March 2000 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 importation and use of Maize Bt-176? If so, what scientific evidence do you believe was insufficient?

- (a) **If the evidence was not sufficient in March 2000, was there sufficient evidence available to Germany 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 importation and use of Maize Bt-176? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

919. Answer 72: In US-65 (31/03/2000), the information submitted by Germany with respect to its safeguard measure, my understanding is that the CA permits to sell seeds limited to 12 tons per year. The reasons stated by Germany were that harmful effects exist for non-target organisms, development of resistance, Bt toxin in the soil and antibiotic resistant gene, so all points were environment related issues.

920. Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the Scientific Committee for Animal Nutrition, the Scientific Committee for Food, the Scientific Committee for Pesticides and the Scientific Committee for Plants (SCP) were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

921. Answer 72a: In my opinion, the scientific evidence presented was sufficient in 2000 and 2003, but I can answer only regarding food safety, and Germany points were related to environmental issues.

**Dr. Andow**

922. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

923. The initial assessment of Event 176 by France provided in EC-158/At.1-3 (10 January 1995, 1 March 1995, 3 March 1995) does not contain the details of the assessment by the French Biomolecular Engineering Committee (BEC).

924. The Opinion of the Scientific Committee on Pesticides (EC-158/At.6\_SCI, 9 December 1996) on Event 176 did not assess the risk of resistance evolution in the target pests because it considered this risk to be an agricultural risk, not an environmental risk. In either event, the Committee felt that resistance management should be fully considered. The Committee also did not assess non-target effects.

925. The Opinion of the SCAN (EC-158/At.4, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on animal health. The Opinion of the SCF (US-64, EC-158/At.5, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on human health.

926. On 23 January 1997, the EC decided to allow the placing on the market of Event 176 (ARG-37).

927. Luxembourg (US-63, 7 February 1997) and Austria (US-52, 13 February 1997) decided to ban the commercialization of Event 176 for reasons covered in questions 69 and 75.

928. The reasoning in the Further Opinion of the Scientific Committee on Pesticides (US-57, 12 May 1997) is false and did not take into account the scientific information known at the time. This is fully discussed in my response to question 69, paragraphs 892-897, and will not be repeated here.

929. In my response to question 69 and question 75, I have concluded that the scientific evidence available to Luxembourg and Austria in February 1997 was NOT sufficient to permit them to undertake an appropriate assessment of potential risks to plants and the environment from Event 176.

930. The Regulatory Committee under Directive 90/220/EC met three times to consider Draft Commission Decisions to require that the temporary prohibitions by Luxembourg and Austria be repealed. The first two meetings (EC-158/At.13, 10 November 1997 and EC-158/At.16, 22 January 1998) resulted in decisions to delay making a decision. The final meeting (EC-158/At.17, 29 April 1998) resulted in no decision (neither to repeal nor not to repeal).

931. On 11 November 1997, an Expert Working Group on Bt resistance management was launched. However, I cannot find any of the proceedings of this Working Group in the materials before the Panel.

932. On 31 March 2000 (US-65), Germany decided to prohibit the unrestricted commercial use of Event 176 in Germany. Germany considered the scientific information for risk assessment to be inadequate in the following areas: effects on non-target organisms, development of resistance, countermeasures against development of resistance, effects of Bt toxin in the soil, horizontal or vertical gene transfer of antibiotic resistance gene, harm to humans from antibiotic resistance gene.

933. On 9 November 2000 (US-66), the SCP provided an Opinion on the German decision. The SCP concluded that the scientific information provided by the German Competent Authority does not alter the original risk assessments on Event 176. The Opinion of the SCP is one possible scientific opinion that can be reached from the information available at the time, but the SCP should have acknowledged that the new information also allows several other scientifically valid opinions that were not justifiable in 1996.

934. On page 3-4 of this Opinion, the SCP deliberates on non-target risks, focusing on three organisms, green lacewing, monarch butterfly and black swallowtail. For the lacewing and monarch, the SCP considered the experiments difficult to interpret and extrapolate to the field. It did not

interpret the swallowtail case. Rather than provide a point by point discussion of the SCP Opinion, I will list a series of valid scientific perspectives and interpretations on the lacewing and monarch studies that illustrate some of the diversity of valid scientific opinion that is not reflected in the SCP Opinion. (1) According to the tiered non-target risk assessment protocols (which are consistent with ISPM 11 and Annex III of the Biosafety Protocol and widely used in the US and Europe), the purpose of laboratory experiments is to expose the organisms to concentrations higher than would be considered typical for the field. By doing so, one reduces the probability of false negative effects. Experimental positives then should undergo additional evaluation. (2) Both lacewings and monarchs were exposed to concentrations of Cry1Ab toxin that would be expected to be higher than typical for the field. Swallowtails were not exposed to high concentrations. (3) Both lacewings and monarchs were adversely affected by Cry1Ab toxin in these laboratory experiments. (4) Additional assessment should have been conducted on lacewings and monarchs to determine the relevance to the field. (5) Cry1Ab is supposed to be a toxin specific to moths and butterflies, but lacewings are not closely related to moths and butterflies. Hence the toxicity spectrum of Cry1Ab toxin is broader than previously expected. (6) Several years later, it has been suggested that Event 176 would have caused significant risk to monarchs had it become a popularly used variety.<sup>129</sup>

935. On page 4 of the Opinion, the SCP deliberates on resistance risk and management, considering two organisms, European corn borer and Mediterranean corn borer. The SCP advised on the establishment of non-Bt refuges adjacent to modified crops but pointed out that, in view of the slow introduction into Europe, crops would be surrounded by natural refuges for some time to come. Rather than provide a point by point discussion of the SCP Opinion, I will list a series of valid scientific perspectives and interpretations that illustrate the prevailing scientific opinion on resistance risk and management in 2000 that is not reflected in the SCP Opinion. (1) The rate of market penetration after initial introduction of Bt maize in the US was the fastest of any crop variety or crop protection technology in the history of US agriculture. Prior to introduction, many predicted that market penetration would be slow. They were wrong. (2) Resistance evolves locally. Thus refuges must be available wherever Bt-maize is locally used. Thus, refuges need to be required from the beginning. (3) Resistance management is the responsibility of each farmer who uses Bt maize. Thus, each farmer should be required to implement refuges. Thus, refuges need to be required from the beginning.

936. On page 5 of the Opinion, the SCP deliberates on toxin release to soil, focusing on degradation processes. The SCP suggests that because protein turnover occurs routinely in soils and degradation of Bt toxin would be expected to degrade at rates similar to other proteins or DNA in the soil, there is no evidence that Bt-toxins will persist in soils and have adverse effects on non-target organisms. Rather than provide a point by point discussion of the SCP Opinion, I will list a series of valid scientific perspectives and interpretations that illustrate some of the diversity of valid scientific opinion that is not reflected in the SCP Opinion. (1) The actual rates and degradation processes for large proteins in soils is poorly understood. (2) Bt toxin loading in maize fields during and after harvest can be substantial. Thus, scale effects are possible. (3) Presently it is known that Bt toxin in the soil can have an adverse effect on earthworms. Whether this translates into an actual risk is not yet known.

937. A close reading (paragraphs 934-936) of the SCP Opinion (US-66, 9 November 2000) suggests that it has not considered all scientific perspectives (and in some cases ignored prevailing scientific opinion). Thus, the SCP Opinion does not invalidate the scientific opinion of Germany.

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<sup>129</sup> NRC 2002. Environmental effects of transgenic plants: The scope and adequacy of regulation. National Academy Press, Washington, DC. 320pp.

938. I conclude that the scientific evidence available to Germany in March 2000 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to plants and the environment from Event 176. The evidence on risks to non-target organisms, including those in the soil could be considered insufficient. Germany could have mustered sufficient evidence to argue that there was an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin, and resistance management measures should be required. There may have been sufficient evidence available to determine what measures should be required, but there probably was insufficient evidence to determine how to implement these measures effectively. This determination by Germany was in direct contradiction to the EC decision. In this case, Germany probably does not need to claim that their safeguard measure is a precautionary one.

939. By 2003, the basis for risk assessment of Bt crops had 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. Annex II of Directive 2001/18/EC is one consequence, but additional regulatory changes have occurred since then. Indeed, knowledge in molecular biology continues to accumulate at remarkably fast rates, and I expect that there will be continued change in regulation in the future.<sup>130</sup> (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.

940. Thus, evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1997 to 2003. This is particularly true in Europe and the United States, and particularly true for the Bt crops.

941. (1) Thus, in 2003, there remains some uncertainty about non-target risks of Event 176, but it could be argued that a risk assessment could be conducted using scientifically justified worst-case assumptions. On the other hand, Germany could reasonably maintain that there is still insufficient information to know which non-target species may be at risk, and therefore it is not possible to conduct an objective risk assessment. The findings in 2003 by Székács and Darvas (EC-158/At.37) – that two protected butterfly species in Hungary, *Inachis io* and *Vanessa atalanta*, might be exposed to Bt corn pollen and suffer higher mortality – certainly suggests that not all of the non-target species at risk to Event 176 have been identified in Europe. (2) As in 1997, Germany could muster sufficient evidence in 2003 to argue that there is an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin in Event 176, and resistance management measures should be required. Moreover, in 2003, Germany should have sufficient evidence to determine what kind of resistance management measures to require. (3) Unlike in 1997, in 2003 Germany could argue that the molecular characterization of Event 176 is insufficient to conduct a risk assessment. (4) It is also possible for Germany to suggest that an additional gene flow risk that needs assessment is contamination of conventional production. It is possible that the scientific evidence to support risk management measures for this risk is presently available.

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<sup>130</sup> Possibilities are suggested in Nap et al. 2004. *Genomics for Biosafety in Plant Biotechnology*. IOS Press, NATO Science Series, Amsterdam.

**Question 73: 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 Germany evaluate the relevant risks to human, plant or animal health, and the environment from the importation and use of Maize Bt-176?**

- (a) **How does the scientific evidence and other documentation submitted by Germany compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

942. Answer 73: Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the Scientific Committee for Animal Nutrition, the Scientific Committee for Food, the Scientific Committee for Pesticides and the Scientific Committee for Plants (SCP) was adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

**Dr. Andow**

943. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

944. With reference to the SPS Agreement, Germany has evaluated relevant risks to plant health. Specifically, Germany has taken into account available scientific evidence (Article 5.2).

945. It is not clear, however, that Germany conducted the risk assessment according to the phytosanitary measures which might be applied. Germany did not present their safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that Germany conducted the risk assessment consistent with Annex A, paragraph 4.

946. Germany did not take into account relevant economic factors (Article 5.3).

947. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because Germany argues that there is a plant pest risk.

948. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

949. It appears that some of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Germany contradict this guidance. My reading of the materials before the Panel is that Germany did not explicitly address this guidance. Specifically I note Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

950. In US-65, Germany made it clear in some points that they are applying a different standard for acceptable risk than reflected in the assessments of the SCAN, SCF, Scientific Committee on

Pesticides in the EC decision (ARG-37) itself. In so doing, Germany fulfilled ISPM 11, Section 3.1 (level of acceptable risk should be expressed).

951. ISPM 11, Section 3.4.6 (prohibition) states "If no satisfactory measure to reduce risk to an acceptable level can be found, the final option may be to prohibit importation of the relevant commodities. This should be viewed as a measure of last resort and should be considered in light of the anticipated efficacy, especially in instances where the incentives for illegal import may be significant." With respect to resistance risk, in 1997 Austria had established the existence of an environmental risk, and required the development of measures to manage this risk to acceptable levels. Germany concurred in 2000. As the EC was not willing to make such a requirement, Germany had no choice but to intervene with its own safeguard measure. In 2000, however, there was probably sufficient scientific evidence to establish what necessary measures should be taken. Scientifically credible suggestions at that time focused on using refuges (maize that was not Bt maize) ranging from 20-30% of the maize grown by any farmer who chose to grow Bt maize. It is unlikely that Germany could have implemented any of these suggestions in time for the 2000 growing season, so I conclude that Germany had fulfilled ISPM 11, Section 3.4.6, at least for the 2000 growing season.

952. The science, documentation and reasoning of Germany are consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Specifically, Annex III, Section 8(f) 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."

**Question 74: Does the scientific evidence and other information submitted by Germany support the adoption of a temporary prohibition on the importation and use of Maize Bt-176? In light of any potential risks identified by Germany, what other risk management options were available in March 2000? What other risk management options are now available?**

**Dr. Nutti**

953. It is important to point out that the main issues raised by Germany were that harmful effects exist for non-target organisms, development of resistance, Bt toxin in the soil and antibiotic resistant gene, so all points were environmental related issues. As far as my knowledge goes, regarding food safety and nutrition issues, the scientific evidence submitted by Germany does not support a temporary prohibition on the import and use of Maize Bt-176.

**Dr. Andow**

954. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

955. The adoption of a temporary prohibition (with <500ha for research purposes) can be justified on the basis of the scientific evidence and other information submitted by Germany.

956. Other risk management options could not have been justified in March 2000. However, had Germany worked to develop its own acceptable resistance management measures, perhaps by 2002 other risk management options would have been possible.

957. Today several alternative risk management options may be available. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. Tolerance strategies are probably inappropriate. One alternate risk avoidance strategy would be to implement country-specific

resistance management measures, to limit planting to a restricted region, and to conduct intensive non-target experiments. This would allow progressive determination of non-target effects.

*Safeguard measure of Luxembourg*

**Question 75:** Given the information before the Panel, including the evaluations undertaken by France (EC-158/at. 1 to 3); the Scientific Committee for Animal Nutrition in December 1996 (EC-158/At.4 and 5); the Scientific Committee for Food in December 1996 (US-64) and in March 1997 (US-58); the Scientific Committee for Pesticides in December 1996 (EC-158/At.6\_SCI) and in May 1997 (US-57); the SCP in September 2000 (US-66); and the European Commission in its Decision of January 1997 (ARG-37), as well as the information submitted by Luxembourg with respect to its safeguard measure (US-63, EC-158/At.9, EC-144), is there any reason to believe that the scientific evidence available to Luxembourg in February 1997 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 importation and use of Maize Bt-176? If so, what scientific evidence do you believe was insufficient?

- (a) **If the evidence was not sufficient in February 1997, was there sufficient evidence available to Luxembourg 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 importation and use of Maize Bt-176? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

958. Before judging the information submitted by Luxembourg, it is important to point out that all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the Scientific Committee for Animal Nutrition, the Scientific Committee for Food, the Scientific Committee for Pesticides and the Scientific Committee for Plants (SCP) were adequate, and the scientific opinion of these committees were that the product could be planted and consumed in Europe.

959. In US-63, Ministerial Decree of 7 February 1997 prohibiting the use and sale of a corn product obtained by genetic transformation, Minister of Health, Luxembourg, considering that the GM product contains the gene conferring resistance to ampicillin and that this circumstance risks having implication for human health, that in the present state of the dossier cannot be properly evaluated and considering that no monitoring program has been implemented with regard to the development of a resistance of the pyralid to the Bt-endotoxin, prohibits the use and sale of the Maize Bt-176.

960. Based on the information presented by the notifiers and by the evaluation of four different scientific committees, my understanding is there is NO reason to believe that the scientific evidence available to Luxembourg in February 1997 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 importation and use of Maize Bt-176.

961. Answer 75a: In my opinion, the scientific evidence presented was sufficient in 1997 and 2003.

**Dr. Andow**

962. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks, specifically the resistance monitoring program.

963. The initial assessment of Event 176 by France provided in EC-158/At.1-3 (10 January 1995, 1 March 1995, 3 March 1995) does not contain the details of the assessment by the French Biomolecular Engineering Committee (BEC).

964. The Opinion of the Scientific Committee on Pesticides (EC-158/At.6\_SCI, 9 December 1996) on Event 176 did not assess the risk of resistance evolution in the target pests because it considered this risk to be an agricultural risk, not an environmental risk. In either event, the Committee felt that resistance management should be fully considered.

965. The Opinion of the SCAN (EC-158/At.4, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on animal health. The Opinion of the SCF (US-64, EC-158/At.5, 13 December 1996) did not address any environmental risk, including environmentally mediated indirect effects on human health.

966. On 23 January 1997, the EC decided to allow the placing on the market of Event 176 (ARG-37).

967. On 7 February 1997, Luxembourg decided to ban the commercialization of Event 176 in Luxembourg (US-63). Luxembourg gave two reasons for this action. 1. A concern that the risks of the bla gene (ampicillin resistance) have not been properly evaluated by the SCAN and the SCF (paragraph 965). 2. That a monitoring program must be implemented to monitor the development of resistance in the target pest to Cry1Ab toxin in Event 176.

968. The Further Opinion of the Scientific Committee on Pesticides (US-57, 12 May 1997) is a major shift in the position of the Committee on Event 176.

969. On page 2-3 of US-57, the Committee states "The Committee stated in its previous report of the 9 December 1996 that resistance management strategies are needed during the years of use of any pesticide, Bt sprays included. The Committee drew attention once again to the need for effective resistance management, including monitoring on agronomic grounds, to prolong the effectiveness of Bt toxin both in conventional sprays and in genetically modified maize. It also felt that the submission of a satisfactory monitoring and resistance management programme should be a requirement for the authorization to use genetically modified maize seeds expressing Bt-toxin." (a) In its 9 December 1996 Opinion, the Committee nowhere made the recommendation that the submission of a satisfactory monitoring and resistance management programme should be a requirement for the authorization to use genetically modified maize seeds expressing Bt-toxin. The acknowledgement that it should be a requirement in this 12 May 1997 Opinion is a major change in the conclusions and recommendations of the Committee. (b) The Committee does not address a significant issue implicit in Luxembourg's statement, which is that resistance risk should be considered an environmental risk. The Committee also seems to be unaware of the position of the US-EPA in 1996, which is that

resistance risk is an environmental risk.<sup>131</sup> This is a critical issue because it determined whether resistance risk could be considered under Directive 90/220/EEC.

970. I conclude that the scientific evidence available to Luxembourg in February 1997 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to plants and the environment from Event 176. Luxembourg could have mustered sufficient evidence to argue that there was an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin, resistance management measures should be required and resistance monitoring is a critical measure. There was probably insufficient evidence available to determine what kind of monitoring should be required. This determination by Luxembourg was in direct contradiction to the EC decision. In this case, Luxembourg probably does not need to claim that their safeguard measure is a precautionary one.

971. By 2003, the basis for risk assessment of Bt crops had 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. Annex II of Directive 2001/18/EC is one consequence, but additional regulatory changes have occurred since then. Indeed, knowledge in molecular biology continues to accumulate at remarkably fast rates, and I expect that there will be continued change in regulation in the future.<sup>132</sup> (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.

972. Thus, evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1997 to 2003. This is particularly true in Europe and the United States, and particularly true for the Bt crops.

973. (1) As in 1997, Luxembourg could muster sufficient evidence in 2003 to argue that there is an environmental risk that the target insects will evolve resistance to the Cry1Ab toxin in Event 176, resistance management measures should be required, and resistance monitoring should be required. Moreover, in 2003, Luxembourg probably had sufficient evidence to determine what kind of resistance management and monitoring measures to require. (2) Unlike in 1997, in 2003 Luxembourg could argue that the molecular characterization of Event 176 is insufficient to conduct a risk assessment. (3) Also in 2003, Luxembourg could argue that an additional gene flow risk that needs assessment is contamination of conventional production. It is possible that the scientific evidence to support risk management measures for this risk were available to conduct an objective risk assessment at that time.

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<sup>131</sup> Matten, S.R., et al., The U.S. Environmental Protection Agency's role in pesticide resistance management, in *Molecular Genetics and Evolution of Pesticide Resistance*, T.M. Brown, Ed., American Chemical Society, Washington, D.C., 1996, pp. 243-253.

<sup>132</sup> Possibilities are suggested in Nap et al. 2004. *Genomics for Biosafety in Plant Biotechnology*. IOS Press, NATO Science Series, Amsterdam.

**Question 76: 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 Luxembourg evaluate the relevant risks to human, plant or animal health, and the environment from the importation and use of Maize Bt-176?**

- (a) **How does the scientific evidence and other documentation submitted by Luxembourg compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

974. Answer 76: Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the Scientific Committee for Animal Nutrition, the Scientific Committee for Food, the Scientific Committee for Pesticides and the Scientific Committee for Plants (SCP) were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

**Dr. Andow**

975. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks, specifically the resistance monitoring program.

976. With reference to the SPS Agreement, Luxembourg has evaluated relevant risks to plant health. However, it is not clear that Luxembourg has taken into account available scientific evidence (Article 5.2). This is not to say that Luxembourg has not taken into account available scientific evidence.

977. It is not clear that Luxembourg conducted the risk assessment according to the phytosanitary measures which might be applied. Luxembourg did not present their safeguard measure as a possible phytosanitary measure. Luxembourg did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that Luxembourg conducted the risk assessment consistent with Annex A, paragraph 4.

978. It is not clear that Luxembourg took into account relevant economic factors (Article 5.3). This is not to say that Luxembourg has not taken into account relevant economic factors.

979. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because Luxembourg argues that there is a plant pest risk.

980. For those risks within the scope of ISPM 11 the risk assessment process may be consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

981. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Luxembourg contradict this guidance. My reading of the materials before the Panel is that Luxembourg did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1

(level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

982. The reasoning of Luxembourg is consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

**Question 77: Does the scientific evidence and other information submitted by Luxembourg support the adoption of a temporary prohibition on the importation and use of Maize Bt-176? In light of any potential risks identified by Luxembourg, what other risk management options were available in February 1997? What other risk management options are now available?**

**Dr. Nutti**

983. It is important to consider that the main issues raised by Luxembourg in order to prohibit the use and sale of the Maize Bt-176 were that the GM product contains the gene conferring resistance to ampicillin and that this circumstance risks having implication for human health, that in the present state of the dossier cannot be properly evaluated and that no monitoring program has been implemented with regard to the development of a resistance of the pyralid to the Bt-endotoxin. So, these are environment related issues.

984. As far as my knowledge goes, regarding food safety and nutrition issues, the scientific evidence submitted by Luxembourg does not support a temporary prohibition on the import and use of Maize Bt-176.

**Dr. Andow**

985. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks, specifically the resistance monitoring program.

986. The adoption of a temporary prohibition can be justified on the basis of the scientific evidence and other information available to Luxembourg. As there are no materials before the Panel containing scientific evidence submitted by Luxembourg, it is not possible to make a determination of the justification for the temporary prohibition based only on submissions from Luxembourg.

987. Other risk management options probably could not have been justified in February 1997. However, Luxembourg could have proposed its own acceptable resistance monitoring measures, which perhaps by 1999 would have been possible to implement.

988. Today several alternative risk management options are available for monitoring for resistance. Although there is still no scientific consensus around the best monitoring method, the cost-efficiency trade-offs are known, and considerable experience has accumulated so that several can be feasibly implemented.

**Maize MON 810 (notification C/F/95/12-02)**

*Safeguard measure of Austria*

**Question 78:** Given the information before the Panel, including the evaluations undertaken by France (EC-159/At.1 and 2); and the Scientific Committee on Plants in February 1998 (CDA-82), September 1999 (US-55), and September 2000 (CDA-86), and the European Commission in its Decision of April 1998 (CDA-81), as well as the information submitted by Austria with respect to its safeguard measure (US-54, EC-159/At.4, EC-144, EC-147, EC-148), is there any reason to believe that the scientific evidence available to Austria in June 1999 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize MON 810? If so, what scientific evidence do you believe was insufficient?

- (a) **If the evidence was not sufficient in June 1999, was there sufficient evidence available to Austria 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 Maize MON 810 (EC-158/At.30-42)? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

989. Answer 78: In EC 159-At 3, Austria informs the applicant that until the uncertainties which have recently arisen during the review have been definitively clarified, Austria is justified in assuming that the cultivation of the product MON810 presents a hazard to human health or the environment. The main points presented were related to the undesired effects on non-target organisms, development of resistance in insects, findings regarding the effects of other Bt plants (cotton in Australia and USA). The document calls particular attention to the study carried out at Cornell University on the Bt maize N4640Bt produced by Novartis, that sparked fierce reactions within the EU. The European Commission, consequently, suspended the authorisation process for the herbicide-tolerant and insect-resistant CryIA(b) Bt maize MON809 produced by Pioneer. Austrian CA also informs that no conclusions have yet been reached by the European Commission regarding the Bt maize plants MON810 produced by Monsanto and Bt-176 produced by Novartis, already approved for cultivation in the EU.

990. The Scientific Committee on Plants (SCP) issued its opinion on the invocation by Austria of Article 16 (safeguard clause) of Council Directive 90/220/EEc with respect to the placing on the market of the GM maize MON 810, and the SCP conclusion was that the information submitted by Austria did not constitute new significant information that was not already considered in its original risk assessment and maintained the previous risk assessment status unchanged. The SCP also concluded that this information does not invalidate its original risk assessments for the other Bt products. I believe the SCP has undertaken a very detailed analysis of the new information and its remarks about the study carried out by Cornell are very detailed, explaining why that study was not conclusive. I completely agree with the SCP opinion and conclusions.

991. Based on the information presented by notifiers and by the scientific evaluation undertaken by France and by SCP, my understanding is there is no reason to believe that the scientific evidence available to Austria in June 1999 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 importation and use of Maize MON 810.

992. Answer 78a: In my opinion, the scientific evidence presented was sufficient in 1999 and 2003.

**Dr. Andow**

993. My response to this question is the same as my response to question 72, except in the following way. It is apparent from the Commission Decision (CDA-81, 22 April 1998) that resistance management and monitoring measures have been proposed by the notifier. However, I could not find in the materials before the Panel a full description of these resistance management and monitoring measures.

994. The Opinion of the SCP (CDA-82, 10 February 1998) does not provide a full description of the proposed resistance management and monitoring measures.

995. Austria decided to prohibit the unregulated commercial use of Mon810 (EC-159/At.4, 10 June 1999). In its explanation for this action, Austria does not provide a full description of the proposed resistance management and monitoring measures.

996. The further Opinion of the SCP (US-55, 24 September 1999) does not provide a full description of the proposed resistance management and monitoring measures.

997. Rather than repeat the arguments in my response to question 72, I summarize as follows. I conclude that the scientific evidence available to Austria in June 1999 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to plants and the environment from Event 176. The evidence on risks to non-target organisms, including those in the soil could be considered insufficient. I cannot provide a scientific judgment about the sufficiency of the scientific evidence on resistance management and monitoring, because a full description of these measures is not available in the materials before the Panel.

998. (1) In 2003, there remained some uncertainty about non-target risks of Mon810, but it could be argued that a risk assessment could be conducted using scientifically justified worst-case assumptions. On the other hand, Austria could reasonably maintain that there is still insufficient information to know which non-target species may be at risk, and therefore it is not possible to conduct an objective risk assessment. The findings in 2003 by Székács and Darvas (EC-158/At.37) – that two protected butterfly species in Hungary, *Inachis io* and *Vanessa atalanta*, might be exposed to Bt corn pollen and suffer higher mortality – certainly suggests that not all of the non-target species at risk to Mon810 have been identified in Europe. However, the toxicity of Mon810 pollen had been determined not to be high, so it is also possible that a worst case risk assessment would have found insignificant risk even for these unknown species exposed to pollen. (2) Unlike in 1999, in 2003 Austria could argue that the molecular characterization of Event 176 is insufficient to conduct a risk assessment. (3) In 2004, Austria suggested that an additional gene flow risk that needs assessment is contamination of conventional production. It is possible that the scientific evidence to support risk management measures is available today.

**Question 79: 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 Austria evaluate the relevant risks to human, plant or animal health, and the environment from Maize MON 810?**

- (a) **How does the scientific evidence and other documentation submitted by Austria compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

999. Answer 79: Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (France) and the Scientific Committee for Plants (SCP) were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

1000. The issues raised by Austria were environmental ones.

**Dr. Andow**

1001. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

1002. With reference to the SPS Agreement, Austria has evaluated relevant risks to plant health. Specifically, Austria has taken into account available scientific evidence (Article 5.2).

1003. It is not clear that Austria conducted the risk assessment according to the phytosanitary measures which might be applied. Austria did not present their safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that Austria conducted the risk assessment consistent with Annex A, paragraph 4.

1004. It is not clear that Austria took into account relevant economic factors (Article 5.3). This is not to say that it has not taken into account relevant economic factors.

1005. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because Austria argues that there is a plant pest risk.

1006. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

1007. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Austria contradict this guidance. My reading of the materials before the Panel is that Austria did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1 (level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

1008. The reasoning of Austria is consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity.

**Question 80: Does the scientific evidence and other information submitted by Austria support the adoption of a temporary prohibition on Maize MON 810? In light of any potential risks identified by Austria, what other risk management options were available in June 1999? What other risk management options are now available?**

**Dr. Nutti**

1009. Answer 80: The main issues raised by Austria in order to prohibit the use and sale of the Maize MON 810 were related to the undesired effects on non-target organisms, development of resistance in insects, findings regarding the effects of other Bt plants (cotton in Australia and USA), and these are environmental related issues.

1010. As far as my knowledge goes, concerning food safety and nutrition issues, the scientific evidence submitted by Austria does not support a temporary prohibition of the import and use of Maize MON 810.

**Dr. Andow**

1011. In my response to this question, I will not address issues related to the antibiotic marker gene and will focus my remarks on environmental risks.

1012. The adoption of a temporary prohibition can be justified on the basis of the scientific evidence and other information submitted by Austria.

1013. Other risk management options may have been justified in June 1999. Because the full resistance management and monitoring measures were not before the Panel, the scientific basis for a concrete discussion of this is not possible.

1014. Today several alternative risk management options may be available. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. Tolerance strategies are probably inappropriate. Because the full resistance management and monitoring measures were not before the Panel, the scientific basis for a concrete discussion of this is not possible.

*Safeguard measure of Italy*

**Question 81: Given the information before the Panel, including the evaluations undertaken by France (EC-159/At.1 and 2); and the Scientific Committee on Plants in February 1998 (CDA-82), September 1999 (US-55), and September 2000 (CDA-86), and the European Commission in its Decision of April 1998 (CDA-81), as well as the information submitted by Italy with respect to its safeguard measure (EC-157/At.1 and 2, CDA-78), is there any reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize MON 810? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in August 1988, was there sufficient evidence available to Italy 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 Maize MON 810? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

1015. Answer 81: At EC 157-At.01, the Italian CA considers illegal the procedure for the placing on the market of certain transgenic foodstuffs, namely BT11 MAIZE, MON810 MAIZE, and MS1 RF1, MS1 RF2 and GT-73 rapeseed oil, MON809 MAIZE and T25 MAIZE, considering illegal because the condition of "substantial equivalence" to existing equivalent foodstuffs required by Article 3, Paragraph 4, of EC Regulation No 258/97 is not met. This document also points the ambiguity of the term "substantial equivalence", that in the opinion of the Italian CA, this concept could be defined differently, given the lack of clarity in the Community legislation. The lead CA informs that 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. The lead Ca does not agree with the technical documentation provided, as far as the comparison of the GMOs and their conventional counterpart is concerned. The document informs that the Superior Institute of Health also came 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 the Italian CA believes that there are inadequacies in the risk assessment procedures. The document also points that the Superior Institute of Health declines to express an opinion regarding the risk of possible "environmental release" of the GMOs in question.

1016. The Scientific Committee on Plants in February 1998 (CDA-82), after examining and considering the existing information and data provided in the dossier, considered that there was no evidence to indicate that the seeds of GM maize MON810, when grown, imported and processed in the manner indicated, are likely to cause adverse effects on human or animal health and the environment.

1017. The Scientific Committee on Plants, in February September 1999 (US-55), concludes that there is no new evidence that would change its previous assessment of GM maize MON 810.

1018. The Scientific Committee on Food, in September 2000 (CDA-86), presents its opinion concerning the submission from the Italian authorities raising concerns from the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, concluding that the information provided by the Italian Authorities does not provide detailed scientific grounds for considering the use of the novel foods in question endangers human health.

1019. The European Commission Decision of 22 April 1998 gives consent for placing on the market the inbred lines derived from maize line MON 810 and any progeny derived from crosses of the product with any traditionally bred maize.

1020. My opinion is that the Italian CA did not provide any scientific information that could change the evaluations done by the scientific committees (Food and Plant). It is important to point out that the information submitted by the applicant, covered all the items needed for the comparison of the GM maize and its conventional counterpart, not only with data on nutrients but also with animal feeding studies. The variation in levels of protein was in the range of natural variation, so the point raised by Italy referring to protein was not correct.

1021. I do not agree with opinion of the Italian CA regarding the ambiguity of the term "substantial equivalence", that, this concept could be defined differently, given the lack of clarity in the Community legislation. The way to proceed for the comparison between the GM and non GM has been presented not only at the Community legislation but also at the OECD consensus documents, FAO/WHO Expert Consultations 1996, 200, 2001 and Codex Alimentarius Guidelines.

1022. My understanding is there is NO reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from e of Maize MON 810.

1023. Answer 81a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Question 82: 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 Italy evaluate the relevant risks to human, plant or animal health, and the environment from Maize MON 810?**

- (a) **How does the scientific evidence and other documentation submitted by Italy compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

1024. Answer 82: All the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (France) and the Scientific Committee for Plants (SCP) and Scientific Committee for Foods were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

1025. Answer 82a: The information and arguments provided by Italy were not in accordance with FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, paragraphs 44 , 45, 48.

**Question 83: Does the scientific evidence and other information submitted by Italy support the adoption of a temporary prohibition on Maize MON 810? In light of any potential risks identified by Austria, what other risk management options were available in August 1988? What other risk management options are now available?**

**Dr. Nutti**

1026. Answer 83: The scientific evidence submitted by Italy do not support a temporary prohibition of Maize MON 810.

**Maize T25 (notification C/F/95/12-07)**

*Safeguard measure of Austria*

**Question 84: Given the information before the Panel, including the evaluations undertaken by France (EC-160/At.1 and 2); the SCP in September 2000 (CDA-75) and September 2001 (CDA-86 and CDA-77), and the European Commission in its Decision of April 1998 (CDA-74), as well as the information submitted by Austria with respect to its safeguard measure (EC-160/At.3 and 5, CDA-76, EC-144, EC-153), is there any reason to believe that the scientific evidence available to Austria in April 2000 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize T25? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in April 2000, was there sufficient evidence available to Austria 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 Maize T25 (EC-158/At.30-42)? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

1027. Answer 84: In EC160 At.03, the Austrian CA prohibits the Maize T25, informing that this event has not been examined under realistic conditions of the use of this herbicide and of correspondent agricultural practice. Neither the notification seeking approval of the placing on the market T25 nor the decision of the European Commission is foreseeing a monitoring programme. It is emphasized that special measures for monitoring the possible spread of pollen to the fields in the surrounding cultivated with conventional maize is missing, and the lack of this monitoring program is crucial. The document also call attention to regional ecological aspects that are not differentiated and the need for further investigations of eventual long term, also secondary, ecological effects.

1028. The SCP, in September 2000 (CDA-75), has considered that there was no evidence to indicate that the use of the GM maize T25 as any other maize, is likely to cause adverse effects on human or animal health and the environment.

1029. The SCP, in September 2001 (CDA-77), gave its opinion on the invocation by Austria of Article 16 of Council Directive 99/22/EEC, regarding the GM maize T25, concluding that the information submitted by Austria did not provide new scientific information to change the original risk assessment carried out on the GM maize T25.

1030. The European Commission Decision of 22 April 1998 gives consent for placing on the market the inbred lines derived from maize line T25 and any progeny derived from crosses of the product with any traditionally bred maize.

1031. Based on the information presented by the notifiers and by the scientific evaluation undertaken by France and by the SCP, my understanding is there is NO reason to believe that the scientific evidence available to Austria in June 1999 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 GM maize T25.

1032. Answer 84a: In my opinion, the scientific evidence presented was sufficient in 2000 and 2003.

**Dr. Andow**

1033. The reasons for Austria's safeguard measure are provided by Austria (CDA-76, EC-160/At.3, 20 April 2000). While the exact statement (unofficial translation) is reproduced below, there are 6 reasons stated. (1) The environmental risks of T25 have not been sufficiently evaluated under realistic conditions. (2) There is no post-commercialization monitoring program. (3) Although harm from pollen transfer to conventional maize production fields is likely absent, the potential for this risk should be monitored. (4) There is no provision for the protection of ecologically sensitive regions. (5) There is a need for regionally differentiated "good farming practice" guidelines to minimize the danger of resistance. (6) There is a need to assess long-term and secondary ecological effects.

The maize line T25 had not been examined under realistic conditions of the use of this herbicide and of correspondent agricultural practice. Neither the notification seeking approval of the placing on the market of T25 nor the decision of the European Commission are foreseeing a monitoring programme.

Furthermore special measures monitoring the possible – mostly regarded as safe – spread of pollen to fields in the surroundings cultivated with conventional maize are missing.

The lack of a monitoring programme regarding long-term effects of genetically modified plants or herbicides can be criticized especially because of the fact that the approval conditions are not foreseeing a protection of sensitive areas (Hoppichler J., Expert Innenbefragung zur Bewertung und Evaluation, "GVO-freier ökologisch sensibler Gebiete", Study on behalf of the Austrian Federal Chancellery, Vienna, 1999).

Furthermore regional ecological aspects are not differentiated: the use of herbicide resistant plants in areas of unavoidable applications of herbicides seems to be useful, if the good agricultural practice minimizes the danger of a resistance development.

Under other ecological – respectively agricultural – conditions the use of herbicide resistant plants such as maize should only take place after further investigations of eventual long-term – also secondary – ecological effects.

1034. Regarding these reasons, (1) is possible grounds for the safeguard measure. (2) is a risk management measure and must be justified by reference to a risk. Austria does not reference any particular risk, so this cannot be grounds for the safeguard measure. (3) is a possible grounds for the safeguard measure. (4) is a risk management measure and must be justified by reference to a risk. Austria does not reference any particular risk, so this cannot be grounds for the safeguard measure. (5) Austria provides no argument that region-specific guidelines are necessary even when the risk of resistance is regionally differentiated. Regarding the risk of resistance, Austria has not differentiated between the risk associated with volunteers and the risk associated with the evolution of resistance in weeds. There is no scientific ground for resistance risk associated with maize volunteers in Europe. Thus the only possible ground for the safeguard measure is the risk of resistance evolution in weeds. (6) presents a class of risks that are possible grounds for the safeguard measure. Thus, I will concentrate on the following four reasons – (1) The environmental risks of T25 have not been sufficiently evaluated under realistic conditions; (3) Although harm from pollen transfer to conventional maize production fields is likely absent, the potential for this risk should be monitored; the specified part of (5), viz., resistance risk in weeds, and (6) There is a need to assess long-term and secondary ecological effects – as possible grounds for the safeguard measure.

1035. Regarding (6), long-term effects are certainly possible, but it is difficult to assess long-term effects in pre-commercial risk assessments. In other words, risks that do manifest on long-term time scales are difficult to predict, and unknown long-term effects must be managed after the fact. Thus, the lack of long-term assessments of unknown effects cannot be considered a reason for the safeguard measure. As no long-term effects have been identified beyond resistance risk, this part of (6) cannot be used to justify the safeguard measure. The remaining part of (6), secondary ecological effects, is a specific case of reason (1) and will be treated together with (1).

1036. Regarding (3), Austria has acknowledged that the possible harm is likely absent, but does not specify concretely the possible harm. It is obvious that Austria cannot be thinking about contamination of conventional production (related to the "coexistence" issue) at this time. If it had considered this, it would not acknowledge that the possible harm is likely absent. As the amended SCP Opinion (CDA-77, 20 July 2001) also does not specific a possible harm, it is difficult to see how this reason can justify the safeguard measure.

1037. Regarding (1), 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. However, Austria does not provide any scientific evidence that such risks may exist for T25 maize. A risk assessment cannot be considered insufficient if all concrete possible risks are addressed. In this respect (1) cannot be used to justify the safeguard measure.

1038. This leaves only resistance risk in weeds as the only possible grounds for the safeguard measure. The amended SCP Opinion (CDA-77, 20 July 2001) does not provide an assessment of this environmental risk. Even though Austria did not provided 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 to assess weed resistance risk and appropriate risk management measures.

1039. Managing resistance risk in maize should be easier than managing resistance risk in oilseed rape, so I would expect that adequate information for a risk assessment could have been made available by 2003, had the parties made concerted efforts to bring it together.

1040. However, as I have stated repeatedly, the evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1997 to 2003. While this has been particularly true for the Bt crops, there has also be a shift for GMHT crops. (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. Annex II of Directive 2001/18/EC is one consequence, but additional regulatory changes have occurred since then. Indeed, knowledge in molecular biology continues to accumulate at remarkably fast rates, and I expect that there will be continued change in regulation in the future.<sup>133</sup> (2) The UK-FSE trials have indicated that there are possible risks to biodiversity associated with herbicide use on GMHT crops. However, for GMHT maize, no adverse effects were found. (3) Contamination of conventional production (related to the "coexistence" issue) is a new issue that could be new grounds for the safeguard measure. It is possible, however, that the scientific evidence to support risk management measures for this risk to conventional maize were available in 2003. Thus, in 2003, Austria could also argue that T25 was inadequately characterized.

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<sup>133</sup> Possibilities are suggested in Nap et al. 2004. *Genomics for Biosafety in Plant Biotechnology*. IOS Press, NATO Science Series, Amsterdam.

**Question 85: 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 Austria evaluate the relevant risks to human, plant or animal health, and the environment from Maize T25?**

- (a) **How does the scientific evidence and other documentation submitted by Austria compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

1041. Answer 85: Environmental issues are not my field, so I cannot judge this point, but all the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (France) and the Scientific Committee for Plants (SCP) were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

1042. The issues raised by Austria are environment related.

**Dr. Andow**

1043. With reference to the SPS Agreement, Austria has evaluated a risk relevant to plant health. However, it is not clear that Austria has taken into account available scientific evidence (Article 5.2).

1044. It is not clear that Austria conducted the risk assessment according to the phytosanitary measures which might be applied. Austria did not present their safeguard measure as a possible phytosanitary measure, and did not conduct the risk assessment according to their safeguard measure. Thus it is not clear that Austria conducted the risk assessment consistent with Annex A, paragraph 4.

1045. Austria did not take into account relevant economic factors (Article 5.3).

1046. The "potential pest" concept of ISPM 11 must first be considered. This is a special case of the general argument in my response to question 6, and that argument holds for this case because Austria argues that there is a plant pest risk.

1047. For those risks within the scope of ISPM 11 the risk assessment process is consistent with ISPM 11. However, the economic assessments called for in ISPM 11 were not conducted.

1048. It appears that much of the ISPM 11 guidance on risk management has not been followed. However, this is not to imply that the actions of Austria contradict this guidance. My reading of the materials before the Panel is that Austria did not explicitly address this guidance. Specifically I note Section 3, S1 (measures should be designed in proportion to the risk), Section 3.1 (level of acceptable risk should be expressed), Section 3.4 (principles), and Section 3.4.6 (on prohibition) were not explicitly addressed.

1049. There is prima facie evidence that Austria did not follow Section 3, S1 (measures in proportion to risk). Resistance risk a long-term risk. Austria probably could have phased in a series of measures that would have allowed planting, but guarded against resistance risk. It is not clear that a prohibition is in proportion to the risk.

1050. The reasoning of Austria is probably consistent with Annex III of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. However, Austria provided little in science documentation to conclude that this was consistent with Annex III.

**Question 86: Does the scientific evidence and other information submitted by Austria support the adoption of a temporary prohibition on Maize T25? In light of any potential risks identified by Austria, what other risk management options were available in April 2000? What other risk management options are now available?**

**Dr. Nutti**

1051. Answer 86: As far as my knowledge goes, regarding food safety and nutrition issues, the scientific evidence submitted by Austria do not support a temporary prohibition on GM maize T25.

**Dr. Andow**

1052. The adoption of a temporary prohibition was probably not justified on the basis of the scientific evidence and other information submitted by Austria. Austria needed to clarify the rationale and provide substantial scientific evidence. A temporary prohibition probably could have been justified at the time because the appropriate scientific information did exist.

1053. Several other risk management options could also have been justified in April 2000. Risk management strategies include risk avoidance, risk mitigation, and risk tolerance. In 2000 mitigation and tolerance strategies were probably inappropriate. Here I consider only the resistance risk. One risk avoidance strategy would have been to allow limited planting in a restricted region. This would allow observing how T25 would be used and enable assessment of the selective pressure on weeds. Another approach would have been to limit use on any particular field to once every 4-5 years. This would reduce selection pressure for a long time, allowing alternative management measures to be developed.

1054. Today the same measures are available.

*Safeguard measure of Italy*

**Question 87: Given the information before the Panel, including the evaluations undertaken by France (EC-160/At.1 and 2); the SCP in September 2000 (CDA-75) and September 2001 (CDA-86 and CDA-77), and the European Commission in its Decision of April 1998 (CDA-74), as well as the information submitted by Italy with respect to its safeguard measure (CDA-78, EC-157/At.1 and 2), is there any reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize T25? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in August 1988, was there sufficient evidence available to Italy 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 Maize T25? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

1055. Answer 87: In EC 157-At.01 and CDA 78, the Italian CA considers illegal the procedure for the placing on the market of certain transgenic foodstuffs, namely BT11 MAIZE, MON810 MAIZE, and MS1 RF1, MS1 RF2 and GT-73 rapeseed oil, MON809 MAIZE and T25 MAIZE, considering illegal because the condition of "substantial equivalence" to existing equivalent foodstuffs required by Article 3, Paragraph 4, of EC Regulation No 258/97 is not met. This document also points the ambiguity of the term "substantial equivalence", that in the opinion of the Italian CA, this concept could be defined differently, given the lack of clarity in the Community legislation. The lead CA informs that 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. The lead Ca does not agree with the technical documentation provided, as far as the comparison of the GMOs and their conventional counterpart is concerned. The document informs that the Superior Institute of Health also came 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 the Italian CA believes that there are inadequacies in the risk assessment procedures. The document also points out that the Superior Institute of Health declines to express an opinion regarding the risk of possible "environmental release" of the GMOs in question.

1056. The SCP, in September 2000 (CDA-75), has considered that there was no evidence to indicate that the use of the GM maize T25 as any other maize, is likely to cause adverse effects on human or animal health and the environment.

1057. The Scientific Committee on Food, in September 2000 (CDA-86), presents its opinion concerning the submission from the Italian authorities raising concerns from the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, concluding that the information provided by the Italian Authorities does not provide detailed scientific grounds for considering the use of the novel foods in question endangers human health.

1058. My opinion is that the Italian CA did not provide any scientific information that could change the evaluations done by the scientific committees (Food and Plant). It is important to point out that the information submitted by the applicant covered all the items needed for the comparison of the GM maize and its conventional counterpart, not only with data on nutrient but also with animal feeding studies. The variation in levels of protein was in the range of natural variation, so the point raised by Italy referring to protein was not correct.

1059. I do not agree with opinion of the Italian CA regarding the ambiguity of the term "substantial equivalence", that this concept could be defined differently, given the lack of clarity in the Community legislation. The way to proceed for the comparison between the GM and non GM has been presented not only at the Community legislation but also at the OECD consensus documents, FAO/WHO Expert Consultations 1996, 200, 2001 and the Codex Alimentarius Guidelines.

1060. My understanding is there is NO reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from GM maize T25.

1061. Answer 87a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Question 88: 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 Italy evaluate the relevant risks to human, plant or animal health, and the environment from Maize T25?**

- (a) **How does the scientific evidence and other documentation submitted by Italy compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

1062. Answer 88: All the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (France) and the Scientific Committee for Plants (SCP) and Scientific Committee for Foods were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessmen..)

1063. Answer 88a: The information and arguments provided by Italy were not in accordance with FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, paragraphs 44 , 45, 48.

**Question 89: Does the scientific evidence and other information submitted by Italy support the adoption of a temporary prohibition on Maize T25? In light of any potential risks identified by Italy, what other risk management options were available in August 1988? What other risk management options are now available?**

**Dr. Nutti**

1064. Answer 89: The scientific evidence submitted by Italy does not support a temporary prohibition on GM Maize T25.

**Maize MON 809 (notification C/F/95/12-01/B)**

*Safeguard measure of Italy*

**Question 90: Given the information before the Panel, including the evaluations undertaken by the Scientific Committee on Plants in May 1998 and September 2000 (CDA-85 and CDA-86), as well as the information submitted by Italy with respect to its safeguard measure (CDA-78, EC-157/At.1 and 2), is there any reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize MON 809? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in August 1988, was there sufficient evidence available to Italy 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 Maize MON 809? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

1065. Answer 90: The SCP, in May 1998 (CDA-85), has considered that there was no evidence to indicate that the seeds of the GM MON809 and progeny derived thereof, when grown, imported and processed in the manner indicated, are likely to cause adverse effects on human and the environment.

1066. In EC 157-At.01 and CDA 78, the Italian CA considers illegal the procedure for the placing on the market of certain transgenic foodstuffs, namely BT11 MAIZE, MON810 MAIZE, and MS1 RF1, MS1 RF2 and GT-73 rapeseed oil, MON809 MAIZE and T25 MAIZE, considering illegal because the condition of "substantial equivalence" to existing equivalent foodstuffs required by Article 3, Paragraph 4, of EC Regulation No 258/97 is not met. This document also points the ambiguity of the term "substantial equivalence", that in the opinion of the Italian CA, this concept could be defined differently, given the lack of clarity in the Community legislation. The lead CA informs that 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. The lead Ca does not agree with the technical documentation provided, as far as the comparison of the GMOs and their conventional counterpart is concerned. The document informs that the Superior Institute of Health also came 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 the Italian CA believes that there are inadequacies in the risk assessment procedures. The document also points that the Superior Institute of Health declines to express an opinion regarding the risk of possible "environmental release" of the GMOs in question.

1067. The Scientific Committee on Food, in September 2000 (CDA-86), presents its opinion concerning the submission from the Italian authorities raising concerns from the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, concluding that the information provided by the Italian Authorities does not provide detailed scientific grounds for considering the use of the novel foods in question endangers human health.

1068. My opinion is that the Italian CA did not provide any scientific information that could change the evaluations done by the scientific committees (Food and Plant). It is important to point out that the information submitted by the applicant, covered all the items needed for the comparison of the GM maize and its conventional counterpart, not only with data on nutrient but also with animal feeding studies. The variation in levels of protein was in the range of natural variation, so the point raised by Italy referring to protein was not correct.

1069. I do not agree with the opinion of the Italian CA regarding the ambiguity of the term "substantial equivalence", that this concept could be defined differently, given the lack of clarity in the Community legislation. The way to proceed for the comparison between the GM and non GM has been presented not only at the Community legislation but also at the OECD consensus documents, FAO/WHO Expert Consultations 1996, 200, 2001 and the Codex Alimentarius Guidelines.

1070. My understanding is there is NO reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from GM maize MON 809.

1071. Answer 90a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Question 91: 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 Italy evaluate the relevant risks to human, plant or animal health, and the environment from Maize MON 809?**

- (a) **How does the scientific evidence and other documentation submitted by Italy compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

1072. Answer 91: All the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (France) and the Scientific Committee for Plants (SCP) and Scientific Committee for Foods were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment).

1073. Answer 91a: The information and arguments provided by Italy were not in accordance with FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, paragraphs 44, 45, 48.

**Question 92: Does the scientific evidence and other information submitted by Italy support the adoption of a temporary prohibition on Maize MON 809? In light of any potential risks identified by Italy, what other risk management options were available in August 1988? What other risk management options are now available?**

**Dr. Nutti**

1074. Answer 92: The scientific evidence submitted by Italy does not support a temporary prohibition of GM Maize MON 809.

**Maize Bt-11 (reference C/GB/96/M4/1)**

*Safeguard measure of Italy*

**Question 93: Given the information before the Panel, including the evaluations undertaken by Great Britain (EC-163(At.1 to 3), the Scientific Committee on Plants in February 1998 (CDA-83), November 2000 (CDA-84), and April 2002 (CDA-35-J), and the European Commission in its Decision of April 1998 (CDA-80), as well as the information submitted by Italy with respect to its safeguard measure (CDA-78, EC-157/At. 1 and 2), is there any reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from Maize Bt-11? If so, what scientific evidence do you believe was insufficient?**

- (a) **If the evidence was not sufficient in August 1988, was there sufficient evidence available to Italy 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 Maize Bt-11? If not, what scientific evidence do you believe was insufficient?**

**Dr. Nutti**

1075. Answer 93: The SCP, in February 1998 (CDA-83), has considered that the import of genetically modified seed (notification C/GB/96/M4/1) carrying the Bt11 event can be considered as safe as utilising seed from non-genetically modified plants.

1076. In EC 157-At.01 and CDA 78, the Italian CA considers illegal the procedure for the placing on the market of certain transgenic foodstuffs, namely BT11 MAIZE, MON810 MAIZE, and MS1 RF1, MS1 RF2 and GT-73 rapeseed oil, MON809 MAIZE and T25 MAIZE, considering illegal because the condition of "substantial equivalence" to existing equivalent foodstuffs required by Article 3, Paragraph 4, of EC Regulation No 258/97 is not met. This document also points the ambiguity of the term "substantial equivalence", that in the opinion of the Italian CA, this concept could be defined differently, given the lack of clarity in the Community legislation. The lead CA informs that 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. The lead Ca does not agree with the technical documentation provided, as far as the comparison of the GMOs and their conventional counterpart is concerned. The document informs that the Superior Institute of Health also came 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 the Italian CA believes that there are inadequacies in the risk assessment procedures. The document also points that the Superior Institute of Health declines to express an opinion regarding the risk of possible "environmental release" of the GMOs in question.

1077. The Scientific Committee on Food, in September 2000 (CDA-86), presents its opinion concerning the submission from the Italian authorities raising concerns from the safety of certain products approved under the notification procedure of Regulation (EC) 258/97, concluding that the information provided by the Italian Authorities does not provide detailed scientific grounds for considering the use of the novel foods in question endangers human health.

1078. The SCP, in November 2000 (CDA-84), considered that there was no evidence to indicate that placing on the market for cultivation purposes of maize line Bt-11 and varieties derived from this line by conventional crosses between Bt11 line and maize lines other than genetically modified ones, was likely to cause any adverse effects on human health and the environment.

1079. The Scientific Committee on Food, in April 2002 (CDA-35-J), presents its opinion on a request to place on the market GM maize Bt-11, and concludes that Bt-11 sweet maize is as safe for human food use as its conventional counterparts. In this document, at item 3.4.2 – Compositional Analysis, the Committee presents a detailed evaluation of the data presented by the applicants and concludes that the one case in which the content of crude protein of the transformed kernel was significant lower than the isogenic control sample, no statistical differences have been observed and concludes Bt-11 sweet maize kernels are substantially equivalent to a non-transformed lines except for the new traits or proteins. In item 3.10 – Nutritional Evaluation, the Committee concludes that the Gm Bt-11, with respect to nutritional evaluation, is substantially equivalent to non-modified maize hybrids. In item 3.12.2 – Toxicological data on the Cry1A(b) protein, the Committee refers to the gastric and intestinal fluid tests, oral toxicological tests and resistance to trypsin. Animal feeding studies were also evaluated and no adverse effects were seen in these studies. I completely agree with the Scientific Committee on Food, and my understanding is that the applicant has conducted all the tests that were necessary for the safety assessment of the Bt-11.

1080. My opinion is that the Italian CA did not provide any scientific information that could change the evaluations done by the scientific committees (Food and Plant). It is important to point out that the information submitted by the applicant covered all the items needed for the comparison of the GM maize and its conventional counterpart, not only with data on nutrient but also with animal feeding studies. The variation in levels of protein was not statically significant, so the point raised by Italy referring to protein was not correct.

1081. I do not agree with the opinion of the Italian CA regarding the ambiguity of the term "substantial equivalence", that this concept could be defined differently, given the lack of clarity in the Community legislation. The way to proceed for the comparison between the GM and non GM has been presented not only at the Community legislation but also at the OECD consensus documents, FAO/WHO Expert Consultations 1996, 200, 2001 and the Codex Alimentarius Guidelines.

1082. My understanding is there is NO reason to believe that the scientific evidence available to Italy in August 1988 was NOT sufficient to permit it to undertake an appropriate assessment of potential risks to human, plant and animal health, and the environment from GM maize Bt 11.

1083. Answer 93a: In my opinion, the scientific evidence presented was sufficient in 1998 and 2003.

**Question 94: 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 Italy evaluate the relevant risks to human, plant or animal health, and the environment from Maize Bt-11?**

- (a) **How does the scientific evidence and other documentation submitted by Italy compare with the relevant international guidelines for risk assessment and analysis identified above?**

**Dr. Nutti**

1084. Answer 94: All the information for the safety assessment of potential risks to human and animal health that was submitted by the applicants and evaluated by the lead CA (Great Britain) and the Scientific Committee for Plants (SCP) and Scientific Committee for Foods were adequate and in accordance to the Codex Guidelines (FAO/WHO Codex Principles for the Risk Analysis of Foods Derived from Modern Biotechnology; FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants; FAO/WHO Codex Annex on Possible Allergenicity Assessment.)

1085. Answer 94a: The information and arguments provided by Italy were not in accordance with FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, paragraphs 44, 45, 48.

**Question 95: Does the scientific evidence and other information submitted by Italy support the adoption of a temporary prohibition on Maize Bt-11? In light of any potential risks identified by Italy, what other risk management options were available in August 1988? What other risk management options are now available?**

**Dr. Nutti**

1086. Answer 95: The scientific evidence submitted by Italy does not support a temporary prohibition on GM Maize Bt-11.

**ISSUE 3**

*Background:* The following questions have the objective of assisting the Panel to determine whether, for the specific biotech products at issue in this dispute, there are significant differences in the risks arising to human, plant or animal health, or to the environment, from the consumption and use of those products and:

- (a) *the consumption and use of those biotech products approved by the European Communities prior to October 1998, taking into account the scientific evidence available at the time of such approvals;*
- (b) *the consumption and use of comparable novel non-biotech products (such as plant products produced by selective breeding, cross-breeding and mutagenesis) for consumption as food or for planting or processing use; and*
- (c) *the consumption and use of foods produced with biotech processing aids, including yeasts, bacteria or enzymes that have been modified using recombinant DNA technology.*

*The experts are not being asked to judge the absolute significance of any differences, but to identify whether differences exist and if so, to what extent, and to assist the Panel to understand the nature and significance of any differences.*

*Furthermore, where there are no significant differences in the potential risks, the objective of the questions is to assist the Panel to determine whether there are scientific or technical reasons for managing any potential risks from the biotech products at issue in this dispute in a different manner than other products (eg., monitoring requirements, agricultural management practices, etc.).*

*With regard to these questions, the experts may wish to comment on the replies by the Parties to the questions by the Panel, in particular the replies to questions 23–26 and 29–32 (replies submitted 16 June 2004).*

**Questions:**

**Products of biotechnology approved by the European Communities prior to October 1998**

**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?**

**Dr. Nutti**

1087. Answer 96: I will not judge any environmental issue, since this is not my area of expertise. However, in the areas of food safety, human and animal nutrition, toxicology and allergenicity, the same approach has been used since 1996 to assess the safety of GM products (FAO/WHO Expert Consultation, 1996). The evolution of the safety assessment process in the last ten years is very much related to the research (which generated a vast bibliography on the subject), discussion and public

perception/awareness developed during the period, when OECD produced several consensus documents and the Codex Alimentarius approved the guidelines for the safety assessment of GM plants and microorganisms. Also, a lot of research has been conducted in different countries (Australia, New Zealand, Japan, USA, European Union etc), in order to improve the whole process. Nowadays, although we have in use (in all steps of the safety assessment) techniques which are more refined, the approach remains essentially the same, fact which is clearly presented in the Codex Alimentarius guidelines.

**Dr. Squire**

1088. From this reviewer's perspective, most of the underlying molecular and biochemical knowledge of the subject has not changed substantially during this period. 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).

**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?**

**Dr. Nutti**

1089. Answer 97: 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.

1090. I will not judge any environmental issue, since this is not my area of expertise. However, in the areas of food safety, human and animal nutrition, toxicology and allergenicity, the same approach has been used since 1996 to assess the safety of GM products (FAO/WHO Expert Consultation, 1996). The evolution of the safety assessment process in the last ten years is very much related to the research (which generated a vast bibliography on the subject), discussion and public perception/awareness developed during the period, when OECD produced several consensus documents and the Codex Alimentarius approved the guidelines for the safety assessment of GM plants and microorganisms. Also, a lot of research has been conducted in different countries (Australia, New Zealand, Japan, USA, European Union etc), in order to improve the whole process. Nowadays, although we have in use (in all steps of the safety assessment) techniques which are more

refined, the approach remains essentially the same, fact which is clearly presented in the Codex Alimentarius guidelines.

1091. According to the WHO document "20 Questions on Genetically Modified Foods", available at [http://www.who.int/foodsafety/publications/biotech/en/20questions\\_en.pdf](http://www.who.int/foodsafety/publications/biotech/en/20questions_en.pdf), the GM foods currently available on the international market have passed risk assessments and are not likely to present risks for human health. In addition, no effects on human health have been shown as a result of the consumption of such foods by general population in the countries where they have been approved. 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.

**Dr. Andow**

**Changes since 1998:**

1092. The main changes that have occurred since 1998 are related to risk assessment methodologies and the evidence needed in a risk assessment. There are some differences between the transgenic crops evaluated and approved before 1998 by the EC and those evaluated later, but these differences are not as great as the changes in risk assessment methodologies and evidence needed. These changes came 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.

1093. As I indicated in previous answers, the basis for risk assessment of transgenic crops had 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.

1094. Thus, risk assessment methodologies and evidentiary standards for what constitutes an objective environmental risk assessment had changed substantially from 1998 to 2005. This is particularly true in Europe and the United States, and particularly true for the Bt crops.

1095. Below, I will sketch some of the changes in non-target risk assessment and resistance risk assessment and management.

**Non-target risk assessment:**

1096. Through 1997, most studies on non-target and biodiversity risks of transgenic plants showed no effect of the transgenic plant (Fitt et al. 1994; Sims 1995; Dogan et al. 1996; Orr & Landis 1997; Pilcher et al. 1997; Yu et al. 1997; EPA 2001; Monsanto Company 2002a, b). The methodological approach used in nearly all of the studies was an indicator species approach similar to the ecotoxicological assessments of pesticides where indicator species are used to extrapolate to risks in the actual environment. Only one laboratory study showed a lower survival of the springtail *Folsomia candida* (Willem) when fed with high concentrations of Bt corn leaf protein (EPA 2001), but the significance of this result is not clear. These indicator species are not usually closely associated with

the transgenic plant tested or the area where the plants are grown. Based on these studies, many scientists believed that non-targets were not significantly at risk.

1097. In 1998, studies by Hilbeck et al (1998a,b) invigorated consideration of non-target risks by reporting an unexpected adverse effect of Bt corn on the predatory green lacewing *Chrysoperla carnea* Stephens. They fed *C. carnea* larvae with Cry1Ab Bt corn-fed prey or a diet containing purified Cry1Ab toxin, and found higher immature mortality compared to controls. These results were surprising because the Cry1Ab toxin is believed to be Lepidopteran-specific, while *C. carnea* belongs to the Neuroptera, an order that is more closely related to the Coleoptera than to the mecopteroid orders including the Lepidoptera. These results have been confirmed by additional studies (Hilbeck et al. 1999; Dutton et al. 2002; Dutton et al. 2003), although the mechanism is still uncertain. These studies suggested that Cry toxins may be less specific than previously believed.

1098. In the years following 1998, publications on non-target risks began to shift from indicator species to the actual (or tangible) potential risks on species that naturally occur in areas where transgenic plants are meant to be cultivated. In early 1999, Losey et al. (1999) suggested that monarch larvae (*Danaus plexippus* L.) suffered higher mortality when feeding on their primary host plant, the common milkweed *Asclepias syriaca* L., dusted with transgenic Cry1Ab Bt pollen. This initial observation was later confirmed by Jesse & Obrycki (2000) and coupled with the realization that ~50% of the monarch breeding habitat is located in the Corn Belt (Wassenaar & Hobson 1998), this triggered concerns that large-scale cultivation of Bt corn would harm the monarch population. Monarch butterflies attract wide interest in the US for multiple reasons, such as their beauty, iconic significance to the public, and spectacular migration over several thousand miles. Stimulated by these results, a group of researchers conducted a series of studies to estimate the actual risk of Bt corn to monarch butterflies (Hellmich et al. 2001; Oberhauser et al. 2001; Pleasants et al. 2001; Sears et al. 2001; Stanley-Horn et al. 2001; Zangerl et al. 2001). Sears et al. (2001) concluded that the risk to monarch populations was insignificant, and in an excellent review, Oberhauser & Rivers (2003) summarized the events and findings associated with these studies. Recent studies (Anderson et al. 2004; Jesse & Obrycki 2004; Dively et al. 2004), however, have revealed a higher toxicity of Bt pollen and anthers than found in previous studies. Although Dively et al. (2004) suggested that the risk to monarchs remains insignificant, a close analysis of the issues may allow other interpretations of risk to monarchs.

1099. This shift to considering tangible risks may have helped identify potential adverse effects to the Federally endangered Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov, Lepidoptera, Lyceanidae). Instead of focusing only on commercial corn fields, dispersal of pollen and the production of corn in wildlife refuges could expose this endangered species to Bt pollen. The 2000 Scientific Advisory Panel (SAP) of the US Environmental Protection Agency (EPA) acknowledged the possibility that this species may come in contact with Bt pollen (see also Andow et al. 1995), and the EPA (2001) required additional assessment of the risks to Karner Blue butterfly.

1100. During 1999, research oriented toward assessing the tangible potential risks associated with soils was published. Saxena et al. (1999) found that Cry1Ab is released into the soil via corn root exudates, where it can persist for at least 350 days (Saxena et al. 2002). These results suggested that Bt corn could possibly affect rhizosphere and soil communities. Later, Zwahlen et al. (2003a) reported that the Cry1Ab toxin in Bt corn litter persisted for at least 8 months. Together these studies showed that long-term exposure of soil organisms to Bt toxins was possible and that the risks of Bt crops on soil ecosystem functioning should be assessed. Zwahlen et al. (2003b) also showed that mortality and weight development of adult and juvenile earthworms, *Lumbricus terrestris* L., were not significantly different when fed Bt or non-Bt corn residues, with the exception that after 200 days, adult Bt corn-fed earthworms had a significant weight loss compared to the non-Bt corn-fed ones.

1101. Non-target risks associated with herbicide-tolerant crops were hardly studied until 2000, when Watkinson et al. (2000) suggested that these crops might adversely effect skylark populations in the UK. A large-scale field evaluation of herbicide-tolerant crops in the UK was established to investigate possible actual effects on non-target species, and results were published in 2003. For the most part, ecological effects propagated from whatever changes in the weed community that resulted from the change in herbicide use. The non-target effects of herbicide tolerant crops have not been studied intensively elsewhere.

1102. In 2004, Andow & Hilbeck (2004) presented the outline of a new risk assessment model for non-target effects of transgenic crops. Implicitly they are proposing to systematize the actual/tangible risk assessment process that has been building since 1999. An important innovation is to select locally occurring non-target species that are most likely to be exposed to a transgenic crop and likely to make significant contributions to the ecological functioning of the local ecosystem.

1103. Non-target and biodiversity risk assessments of transgenic plants continue to be improved. While indicator species continue to be used in many risk assessments, there is a trend towards assessing actual/ tangible risks involving species that naturally occur in areas where transgenic crops will be planted. In the future it will continue to be important to assess not only the effects of the transgenic plant itself but also the effects associated with changes in agricultural practices. In addition, although they may be difficult to develop and verify, effective methods for biodiversity assessment have not yet been developed.

#### Resistance risk assessment and management:

1104. The evolution of pest resistance to pest control measures has been known for nearly 100 years, but it became a significant problem after World War II, when modern, intensive agricultural technologies proliferated, resulting in strong uniform selection over large areas. About 536 species of arthropods, 60 genera of plant pathogenic fungi, and 174 weed species have evolved resistance to pesticides (Eckert 1988; WeedScience.org 2003; Whalon et al. 2004), and resistance to Bt toxins has been documented in >17 insect species (Tabashnik 1994, Huang et al. 1999). Interestingly, virologists remain unconvinced that resistance will evolve to transgenic virus resistant crops (Tepfer 2002), however, despite some disagreement (reviewed in Tabashnik 1994), entomologists and weed scientists agree that resistance evolution is a real risk for which some management is desirable (NRC 1986).

1105. At the beginning of the 1990s, it had proven difficult to implement effective resistance management for most pesticides. Indeed, there was pessimism that a high-dose/ refuge resistance management system could ever be implemented for insecticide resistance management because a high dose could not be reliably maintained (Roush 1989; Tabashnik 1989). With the advent of transgenic Bt crops, hopes were renewed (Gould 1994; Roush 1994), but it was not clear that a high-dose/ refuge strategy would delay resistance enough with a reasonably sized refuge (Comins 1977).

1106. In a series of simple simulations based on Comins (1977) early work, Alstad & Andow (1995) showed that the high-dose/ refuge strategy could delay resistance in European corn borer to Bt corn for more than 30 years with a 50% non-Bt corn refuge. Subsequent research suggested that smaller refuges would also substantially delay resistance, proving that effective resistance management was possible theoretically (Gould 1998; Shelton et al. 2000).

1107. The focus shifted to practicalities. Could an effective resistance management strategy be implemented? In the US, this question was answered through a series of decisions made by the EPA. In early 1995, the EPA registered Bt potato, and although resistance risk was recognized, no

resistance management was required. By the end of that year, EPA issued conditional registrations and required the development of resistance management for all subsequent Bt crops (Matten et al. 1996). Conditional registrations were used to motivate the development and implementation of a scientifically justified resistance management strategy.

1108. The key issues in 1995 were how large a refuge was needed, did the refuge need to be spatially structured relative to the Bt fields, and could the refuge be managed to limit pest losses? Aspects of some of these questions remain unresolved today. In Australia, Bt cotton did not provide a high-dose against the key pest, cotton bollworm *Helicoverpa armigera* (Hübner), and growers and researchers agreed to require 70% refuges to make the likelihood of resistance remote (Fitt 1997). In the US, Bt cotton provided a high-dose against the key pest, cotton budworm *Heliothis virescens* (F.), but not against another important pest, *Helicoverpa zea* (Boddie), however, refuge requirements were set at 4% unsprayed or 20% sprayed refuge outside of the Bt field with minimal requirements of spatial structure. In 2001, spatial structure requirements were added for Bt cotton along with other modifications. These initial requirements and changes represent a compromise among various interests, although science played a significant role.

1109. Resistance management requirements for Bt corn developed with strong scientific input. Early in 1997, the USDA regional research committee NC-205 reviewed model results and information on the ecology of European corn borer and suggested to registrants and the EPA that a 20-25% refuge was needed near all Bt corn fields (Anon. 1998). Research results supporting this recommendation were published in the ensuing years (Onstad & Gould 1998; Hunt et al. 2001; Bourguet et al. 2003). One of the key results was a bioeconomic model suggesting that a 20% refuge would be nearly optimal for growers who consider the trade-off between the immediate costs of the refuge and delayed costs of resistance failures (Hurley et al. 2001). Canada required a 20% refuge within 0.5 miles (~800 meters) of Bt corn in 1998, and during 1999 a consensus was reached in the US and the EPA required a 20% refuge within 0.5 miles of Bt corn for the 2000 growing season and thereafter.

1110. Several scientific issues remain unresolved. Understanding the mechanisms of resistance is necessary to tailor resistance management to the particular system, but these are just beginning to be revealed for Bt crops (Gahan et al. 2001). The details of adult movement may play a key role in the evolution of resistance (Caprio 2001; Ives & Andow 2002). Limited dispersal of adults from natal fields (Comins 1977), pre- versus post-mating adult movement, and male versus female movement (Ives & Andow 2002) may have significant effects on models of resistance evolution, and estimating these movement rates in the field is challenging. Farming practices, such as crop rotation (Peck & Ellner 1997), management of the refuge (Onstad et al. 2002; Ives & Andow 2002), and rational approaches to pest management, may affect the rates of resistance evolution. Significantly, a consensus for managing low-dose events has yet to emerge, and scientific analysis of this problem is incomplete. For example, Australia implemented 70% refuges for one low-dose event, while the US has used 20% refuges for both high- and low-dose events.

1111. Monitoring for the occurrence and frequency of resistance and methods to improve compliance to resistance management requirements among growers are areas of current research. The key monitoring problem is how to estimate resistance when it is rare and recessive. One promising approach is the F<sub>2</sub> screen (Andow & Alstad 1998; Andow & Ives 2002; Stodola & Andow 2004). It is a genic screen and works by inbreeding isofemale lines so that recessive phenotypes are expressed in the F<sub>2</sub> generation, when they can be screened. If mated females are collected from natural populations, each carries four haplotypes (two of her own and two of her mate's) and only 250 female lines need to be screened instead of 10<sup>6</sup> field-collected individuals. The F<sub>2</sub> screen has been used for several species (Bentur et al. 2000, Bourguet et al. 2003; Génissel et al. 2003). Other genetic and

phenotypic methods have been used on some cotton pests (Gould et al. 1997; Tabashnik et al. 2000), but usually phenotypic screens will have lower sensitivity and higher cost than genic screens (Andow & Ives 2002). Improving compliance will require a combination of bioeconomic modeling, surveys of grower behavior and motivations, and development of effective educational materials.

1112. Our understanding of resistance risk and management continues to evolve. Presently, none of the Bt crops now used has suffered a resistance failure despite widespread use. While this may be due to good fortune, in some cases, such as Bt cotton in Australia, resistance management must have been crucial to avoiding failure, and in other cases, such as Bt cotton in Arizona, US, other factors must also be important (Tabashnik et al. 2003). Interestingly, relatively little research has focused on weed resistance to herbicides used with the herbicide tolerant crops. This problem has been treated as a theoretical herbicide resistance problem (Gressell et al. 1996), but with recent reports of weed resistance (WeedScience.org 2003), this may change.<sup>134</sup>

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**Dr. Snow**

1113. I conclude that 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

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predecessors. Adopting these herbicide-tolerant crops could lead to greater dependence on these and other herbicides (see Part I and Friesen et al. 2003).

1114. 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. Although another variety of oilseed rape with resistance to the herbicide imidazolinone is widely grown in Canada and Australia, the relatively minor problems associated with its use could be compounded with the addition of transgenes that provide more resistance to more herbicide modes of action. The Canadian regulatory system treats all herbicide-tolerant crops in a similar manner, regardless of whether they are transgenic or not, and it appears that little or no effort has been made to avoid gene flow among herbicide-tolerant crops, volunteers, and weeds. The EC approach differs dramatically in that EC considers possible long-term effects that might arise from the combination or successive use of herbicide-tolerant crops.

1115. Application rates of glufosinate and glyphosate are expected to increase greatly if these herbicide-tolerant crops are adopted by farmers. In Europe, scientists are still investigating the question of whether these types of changes in agricultural practices could have unwanted effects on farmland biodiversity, relative to ongoing effects of conventional, nontransgenic crops. This research started during 1998-2003 and findings have recently begun to appear in scientific publications (e.g., Watkinson 2000, Firbank 2003, Squire et al. 2003). Also, in the context of Europe, gene flow between transgenic oilseed rape crops and non-transgenic crops is perceived as an economic problem due to newly introduced labelling requirements. Research is under way to develop effective strategies for "coexistence" of transgenic and conventional crops.

1116. As discussed above, scientific experts in the EC have concluded that potential problems related to gene flow should be monitored after commercialisation, and that farmers should be educated about appropriate stewardship and management of herbicide-tolerant crops. The scientific committees of certain Member States (e.g., France) have argued that more research is needed to determine whether problems related to gene flow can be mitigated or avoided after these crops are grown on a large scale. In Canada and the US, however, no measures have been taken to avoid these problems. Glyphosate-tolerant oilseed rape is grown very widely in western Canada, where this herbicide is used in low-till and no-till weed management.

1117. Here, I have focused on herbicide-tolerant crops. Other scientific studies have raised valid questions about the persistence of Bt toxins in the environment and their potential to harm nontarget insects (e.g., Losey 1999, Stotzky 2001, Hilbeck 2001). To my knowledge, these questions largely have been resolved by subsequent scientific findings with regard to the crops that are the focus of this dispute.

1118. Furthermore, I do not know of any studies or scientific evidence showing that the process of creating transgenic plants is inherently more risky than other methods used in conventional breeding, including mutagenesis and embryo rescue following wide crosses.

1119. 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**

1120. The argument presented in Notes 11-12 is 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. Examples of where knowledge has increased substantively are –

- Spread and persistence of some crops plants, notably oilseed rape.
- The important role of insects as carriers of pollen over the landscape.
- The importance of the weed flora to in-field biodiversity and food webs.

**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 (eg, 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?**

**Dr. Nutti**

1121. Answer 98: As I already answered in question 1, it will be necessary a combination of several factors for a DNA transfer from plants to microbial or mammal cells to occur, which is the main argument raised in this dispute against the products using antibiotic resistance marker genes.

1122. It is important to point out that according to FAO/WHO (2000), DNA transfer 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.

1123. 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. 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.**

1124. The transfer of marker genes which confer resistance to kanamycin, ampicillin and streptomycin to bacteria in the human gut is unlikely to present a significant health impact since bacteria resistant to these antibiotics are already spread all over or are naturally found in the human gastrointestinal tract (Smalla et al., 1993; Calva et al., 1996; Shaw et al., 1993; Smalla et al., 1997).

Besides, kanamycin/neomycin and streptomycin are rarely used for humans due to their collateral effects (WHO 1993).

1125. 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 (eg, 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.

1126. I will not comment on any environmental issue, since this is not my area of expertise. However, in the areas of food safety, human and animal nutrition, toxicology and allergenicity, the same approach has been used since 1996 to assess the safety of GM products (FAO/WHO Expert Consultation, 1996). The evolution of the safety assessment process in the last ten years is very much related to the research (which generated a vast bibliography on the subject), discussion and public perception/awareness developed during the period, when OECD produced several consensus documents and the Codex Alimentarius approved the guidelines for the safety assessment of GM plants and microorganisms. Also, a lot of research has been conducted in different countries (Australia, New Zealand, Japan, USA, European Union etc), in order to improve the whole process. Nowadays, although we have in use (in all steps of the safety assessment) techniques which are more refined, the approach remains essentially the same.<sup>135</sup>

**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, arising from the consumption or use of these products compared to those products of biotechnology approved by the European Communities prior to October 1998?**

**Dr. Nutti**

1127. Answer 99: In my opinion, 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.

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<sup>135</sup> References:

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1128. 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.

1129. According to the WHO document "20 Questions on Genetically Modified Foods", available at [http://www.who.int/foodsafety/publications/biotech/en/20questions\\_en.pdf](http://www.who.int/foodsafety/publications/biotech/en/20questions_en.pdf), the GM foods currently available on the international market have passed risk assessments and are not likely to present risks for human health. In addition, no effects on human health have been shown as a result of the consumption of such foods by general population in the countries where they have been approved. 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.

**Dr. Andow**

1130. There are some biotech products at issue in this dispute for which one committee or another has determined that there were no significant risks. I have taken issue with some of these determinations in my previous responses. 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.

1131. This disagreement provides the only rationale for monitoring potential adverse effects. If all parties agreed that there were no significant identified risks, there would be no need to consider monitoring for potential adverse effects.

1132. Unanticipated effects could arise from a transgene by any of the following mechanisms: new ORFs, insertional mutagenesis, post-transcriptional or post-translational processing, other pleiotropy or epistasis, and gene-by-environment interaction. New ORFs and insertional mutagenesis can be addressed through molecular characterization of all transgene loci. Post-transcriptional or post-translational processing can be addressed to the most part by molecular and biochemical analysis. Other pleiotropy or epistasis and gene-by-environment interaction cannot be addressed without extensive planting in the field.

1133. All of these possible sources of unanticipated effects are equally likely to occur for plant biotech products before 1998 and plant biotech products after 1998. It is possible that more recent events (after 2001) are less likely to have such unanticipated effects than those prior to 1998. The main difference between them is that new ORFs, insertional mutagenesis and post-transcriptional and post-translational processing was not looked for very much before 1998, while after 2001, the methods and standards have become increasingly targeted to assess these possibilities. This provides a rationale for monitoring.

**Dr. Snow**

1134. 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 has identified potential risks.

1135. With regard to environmental effects, if this question pertains only to products that have no identified environmental risks, then there is no scientific rationale for obligatory monitoring. One could argue that possible risks may have been overlooked prior to marketing, but it is not logical to require monitoring for every transgenic crop, regardless of its phenotypic traits. Rather, this might be carried out to reassure the public that long-term studies are being conducted, or to ensure that labelling thresholds for non-GM products are not exceeded.

1136. If gene flow from transgenic crops is viewed as a form of genetic "contamination", then determining the extent to which it occurs after deregulation could be seen as a socio-economic reason for monitoring. In this context, scientific knowledge that was gained after 1998 is relevant because many studies showed that gene flow occurs more widely and more quickly than was previously anticipated (see above).

1137. In reality, 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.

**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?**

**Dr. Andow**

1138. A parallel argument to my response to question 99 holds for this question.

**Dr. Snow**

1139. See Answer 99 because the same principles apply.

**Dr. Squire**

1140. Following the points made in answer to Q.97, 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.

**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?**

**Dr. Andow**

1141. None of the products approved by the EC prior to October 1998 have been mitigated in any manner by the EC because no mitigable risks have been reported and verified. Should any of the biotech products cause a verifiable risk, this would be sufficient argument for mitigating them differently.

1142. However, as indicated in my response to question 97, 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.

**Dr. Snow**

1143. Several of the products that were approved before 1998 are the same as those in this dispute or are very similar. These include the following, as listed in the US June 16, 2004 documents:

**Oilseed rape (= Swede rape)**

Glufosinate-tolerant, male sterile (MS1,RF1); C/UK/94/M1/1  
Glufosinate-tolerant (Topas 19/2); C/UK/95/M5/1

**Maize**

Bt-176, glufosinate-tolerant; Ceiba-Geigy; C/F/94/11-03  
Bt cry1A; MON 810; Monsanto; C/F/95/12-02  
Bt-11; glufosinate-tolerant with Bt cry1Ab; C/UK/96/M4/1  
Glufosinate-tolerant (T25); AgrEvo; C/F/95/12-07

**Chicory**

Glufosinate-tolerant, male sterile; C/NL/94/25

1144. 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.

1145. In summary, 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).

1146. In the case of oilseed rape that was approved for import and processing only, I cannot identify new scientific reasons for different mitigation plans (e.g., using covered vehicles when transporting large quantities of seeds).

**Dr. Squire**

1147. The answers follow from the responses above to questions 97 and 100. 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.

1148. 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. Early research using GMOs in small parcels of land could not possibly have generated the knowledge that pollen from large fields of oilseed rape is moved widely in the landscape. Moreover, the present guidelines about pollen barriers, e.g. a strip of flowering crop between the GM field and non-GM recipient field, may have to change if the proportion of GM fields in the landscape increased to, say, 50%.

**Comparable novel non-biotech products, (such as plant products produced by selective breeding, cross-breeding and induced mutagenesis)**

**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.**

**Dr. Nutti**

1149. Answer 102: It is important to point out that usually the novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis have not been subjected to any extensive safety assessment as the plants derived from the Recombinant-DNA technology have been. 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.

**Dr. Andow**

1150. Comparable non-biotech products from plant breeding: Regarding the differences between conventional breeding and so-called "molecular breeding," none of the parties fully represent the comparison accurately. Breeding involves two important processes: (1) finding and introducing usable genetic variation into the breeding population and (2) improving and selecting desired varieties from the breeding population. The methods used will depend on the crop, with major differences for

clonal species, such as potato and banana versus sexual species, such as oilseed rape, maize and soybean. Among the sexual species, there are major differences in breeding methods between outcrossing species, such as maize and oilseed rape, and inbreeding species, such as soybean, and wheat.

1151. Transgenesis is a process to introduce genetic variation into the breeding population. Transgenesis is not a process for finding genetic variation or for selecting and improving varieties. Thus, there is no such thing as molecular breeding. Transgenesis is merely a part of the breeding process.

1152. It would seem important then to determine a comparable conventional counterpart to transgenesis for introducing genetic variation into the breeding population. A comparable conventional process would be one typically used in breeding programs rather than one rarely used. If possible a comparable conventional process would introduce genetic variation during a similar or analogous step in the breeding process. The breeding process can eliminate considerable genetic variation, so variation introduced very early in the breeding process, will be screened and selected multiple times by multiple breeding programs prior to use, while variation introduced much later in the process will receive fewer screenings and possibly no additional selection.

1153. Several other methods exist to introduce genetic variation into the breeding population. These include wide crossing, crossing or using unadapted material, mutagenesis, and recombining diverse adapted material. Wide crossing involves crosses with different species. Unadapted material are plants of the same species (or subspecies) that have not previously been used in breeding programs. Adapted materials are those that have already been used to produce modern conventional varieties. There is a range of "adaptedness" even within these materials, with some breeding programs relying primarily on the most recently used popular varieties and other programs reaching further back in time for older varieties as the source of genetic variation.

1154. Crossing methods are difficult to use routinely for clonal and inbreeding species. For example, in potato, genetic variation is conventionally introduced by growing plants to flowering and crossing material, or via deliberate or spontaneous mutation during cell culture. The crossing methods take a longer time to produce usable varieties. For example some characters have not been incorporated in usable varieties despite over 30 years of work. Cell culture is essential for producing new potato seed stock, so it is a routine way to introduce genetic variation and transgenesis introduces genetic variation in cell culture. Thus for transgenic potatoes, the most relevant conventional comparison is deliberate or spontaneous mutation in cell culture.

1155. For inbreeding species, such as soybean and dry bean, variation can be introduced by hand pollinations (usually involving adapted material), or by using new collections because inbreeding lines will breed true to type. In some cases it is possible to create situations where the inbreeding plant will outcross at higher rates. In this way, it is possible to introduce variation using crossing methods. However, because the crossing methods typically are difficult, most programs do not use them very much. All programs rely on evaluating collections (sometimes an old collection in a new environment) for new, usable genetic material. For these species, transgenesis is a new and powerful method for introducing genetic variation directly into the adapted modern varieties, thereby avoiding the expense of crossing and the arbitrariness of collections. Thus, it is not clear that there is a comparable conventional method.

1156. For outcrossing species, such as maize and oilseed rape, there are many means to introduce genetic variation. For modern maize breeding, however, wide crosses and crossing with unadapted materials are not used in the vast majority of breeding programs in the world. Many public sector

programs devote some resources to these, but they are viewed as long term breeding efforts that may produce improved populations over decadal periods. These improved populations may or may not be picked up by other breeding programs for introduction into their unimproved breeding populations. By far the most common method for introducing new genetic variation into a maize population for breeding is recombination of adapted material. However, dwarfing even this method is "backcrossing," which is a method for introducing a specific genetic trait into an adapted inbred line. Both conventional and transgenic traits are introduced into adapted inbred lines via backcrossing. However, for maize, recombination of adapted material is probably the most relevant conventional comparison to transgenesis.

Types of risks:

1157. As found by all scientific panels addressing this issue, 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.

Nature or magnitude of risks:

1158. Within these kinds of risk, there are new risks of biotech plants.

1159. The EC is correct in pointing out that both biolistic and Agrobacterium-mediated transformation typically results in multiple transgene loci, typically with complex structure.<sup>136</sup> Unlike bacterial transformation, where what is intended to be inserted typically is inserted just as intended, this is not typical using the present plant transformation methods. One of the main concerns arising from this are new open reading frames (ORFs). An ORF is a DNA sequence that could theoretically produce a protein. A new ORF could theoretically produce a new protein, which could cause or influence a new risk.

1160. The EC and the US is correct to identify insertional mutagenesis as another outcome of transgenesis that is new to breeding, and could cause or influence a new risk.

1161. Canada is incorrect to claim that transgenesis allows more precise control than selective breeding. First both transgenic and conventional varieties undergo some level of selective breeding, so the comparison is inappropriate. More importantly, transgenesis allows control over the intended result (and in this sense, there is no dispute that transgenesis allows more precise control), but the actual result is often different.

1162. The US, Canada and Argentina are correct to say that translocations and other genomic disruptions can occur in conventional breeding. However, these genomic disruptions are normally rare in conventional breeding. Moreover, their frequency of occurrence is much higher when genetic variation is introduced using wide crossing than the more typically used recombination of adapted materials.

1163. Thus, the magnitude of ORFs and insertional mutagenesis introduced by transgenesis is higher than that introduced by recombination of adapted materials. It also may be higher than deliberate or spontaneous mutation in cell culture, although this is less certain.

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<sup>136</sup> Please refer to additional detail in my response to question 9.

1164. There is considerable research being conducted to understand and control the transgene insertion process, and it is likely that over the next decade technical improvements will alter these concerns.

1165. Some of the ORFs and extraneous transgene loci can be eliminated by independent assortment for outbreeding plants, such as maize. This is less possible for inbreeding plants and clonal plants.

1166. Nearly all risks associated with novel toxins (e.g., all Bt crops) introduced into crop plants are new risks. While there are comparable risk assessment models for assessing these risks (toxins in plants), and the risks of the particular toxins may have been investigated outside the plant, the fact that the plant is used as the delivery vector for the toxin and the precise expression patterns in the plant mean that new species are exposed in new ways. These are new risks.

1167. Other risks of a new nature have been identified in some of my responses to the other questions.

1168. The intended use of the product does not affect the nature of the risk, but it does affect the risk quantitatively. This is an effect of scale. For example, risks associated with releases for processing are smaller than the same risks for the same product for planting and other use. It is not clear how much smaller, but in some cases (biodiversity risks of GMHT crops), it could be nil for processing uses while it at the same time is was substantial for cultivation uses.

1169. Risks may change quantitatively from the combination of transgenes, because it will not be possible to maintain seed supplies of all possible combinations of traits. Suppose a crop, such as cotton, was available mainly with both a GMHT gene and at Bt gene. Suppose the insect pest is a sporadic one on cotton, but a major one on maize. Farmers would have less incentive to use the Bt gene, but may have a strong desire to use the GMHT gene. This would lead to an over use of the Bt gene in cotton, increasing resistance risk both in cotton and maize.

### **Dr. Snow**

1170. In my opinion, and only in reference to environmental risks, the biotech products in this dispute could be considered as different from non-biotech products in the some cases, such as:

- Pesticide-producing crop plants –
  - Non-biotech maize does not produce proteins that are toxic to insects, including pollen that can kill lepidopterans (butterfly and moth larvae), whereas Bt maize does (especially Bt-176, see NRC 2002). Here, one concern is that drifting maize pollen could land on the food plants of susceptible, non-target insects and kill them. Although this risk has largely been discounted as the result of several investigations in the USA, it is still a question that scientific advisory panels must consider for all Bt crops. Other non-target effects of Bt maize also require evaluation, including possible unwanted effects on soil invertebrates, pollinators, ladybird beetles, and other beneficial insects.
- Herbicide-tolerant crop plants with feral (volunteer) populations or wild relatives that can hybridise with the crop:
  - See answers to Question 6 and others. Glyphosate-resistance has not been introduced into crops using non-transgenic methods, so this particular trait is

linked to novel transgenic products. Because glyphosate is used so extensively, weedy *Brassica rapa* populations that acquire the transgene for glyphosate resistance might become more common in and around farmers' fields. In the future, this process could occur many types of crops/weed systems where weedy relatives of crops (e.g., weedy rice, chicory, carrot, sunflower, sorghum, sugar beet, turf grass, etc.) are controlled using glyphosate. Also, the herbicide glyphosate provides certain environmental benefits (such as facilitating no-till agriculture and replacing more toxic herbicides) that could be lost if there is extensive gene flow to volunteers and wild relatives, and if over-use of the herbicide selects for other glyphosate-resistant weeds. These problems are possible with all types of herbicide-tolerant crops, unique features of glyphosate make it a special case.

**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?<sup>137</sup> If so, what means of risk mitigation might be envisaged?**

**Dr. Nutti**

1171. Answer 103: My understanding is that plants that were developed using induced mutagenesis should be subjected to the same risk assessment used for plants produced from the Recombinant-DNA technology. In my opinion, crop varieties that have been developed using induced mutagenesis can pose the same risk to human or animal health and, up to now, they have not been properly assessed. I believe that guidelines should be developed for the safety assessment of these plants, and that the FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants could be used as baseline.

**Dr. Andow**

Risk and "contamination":

1172. This will depend on the scale of release and the nature of the adverse effect.

1173. Risk is a combination of exposure and an adverse effect. Exposure will be determined by the properties of the crop plant and the quantity of each planted (scale of release). There is little evidence to suggest that ceteris parabis, gene flow will be greater from a transgenic variety than a conventional one.<sup>138</sup>

1174. The definition of an adverse effect entails specification of who or what will be affected and how is this effect considered adverse. Thus an organic farmer may find contamination adverse because it removes his or her product from the organic food stream. This could be considered adverse because of economic loss and loss of quality of life or livelihood.

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<sup>137</sup> 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/>.

<sup>138</sup> Some transgenic plants may have lower levels of gene flow, such as the male-sterile oilseed rape varieties.

1175. There has been little discussion and less agreement over the nature of the adverse effects of contamination. For example, there can be economic and livelihood harms associated with the perception of adverse effects. It is not clear to me that there is agreement that these kinds of perceived risks should be considered.

1176. For the example in paragraph 1174, the risk associated with biotech crops is substantially greater than the risk associated with any of the conventionally produced varieties. However, this is largely due to the definition of the harm.

1177. Although there is little data to support this hypothesis, I would suggest 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. In general, "contamination" will be a spread process for which there is no advantage of the transgene in the invaded/ contaminated habitat, but there is an advantage in the habitat of origin. In these cases, spread scales with the length of the boundary between the two habitats (not the area of either) and linearly with the square of time.<sup>139</sup> However, at very small spatial scales, it is likely that managers will be able to take greater care in limiting accidents, resulting in lower contamination rates.

#### **Dr. Snow**

1178. The question of whether any of the biotech products in this dispute poses a substantially greater risk due to "contamination" than non-biotech products depends on what types of risks each party is trying to avoid (see Part I). If one considers that standard thresholds have been established to allow the "coexistence" of biotech and non-biotech crops in Europe, then by definition contamination is an issue for all biotech products. Very low levels of contamination can be detected using DNA markers, with the result that contamination, also known as "adventitious presence," has been detected routinely in non-biotech products. Labeling and traceability have not been required for novel non-biotech products such as those that were developed using mutagenesis, nor would any high-resolution identifying markers be available for these types of crops.

1179. Another way to answer this question is to focus on the characteristics of biotech crops – their phenotypes – rather than the mere presence of transgenes. This is more appropriate if the goal is to avoid direct or indirect harms to human, plant or animal health, or the environment. 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. See Part I and answers to Question 6 and others above for examples of risks that are greater in certain GM crops as compared to their non-GM predecessors.

#### **Dr. Squire**

1180. A certain level of impurity – through one crop type growing within or giving pollen to another type – seems to have been generally accepted for many years. Such impurity varies greatly with crop type: it is common in oilseed rape, and less common in beet and maize, for example. Where the nature of the yield is different, e.g. for high erucic oilseed rape (HEAR), impurities are kept to acceptably low values by using registered growers who are familiar with the crop and with the requirements for separation, and many of whom farm in a restricted part of the country. There are clear criteria for the acceptable presence of HEAR in non-HEAR oil. Outside specialist varieties such

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<sup>139</sup> This is much slower than for species invasions, which scale on the square root of the area occupied and linearly with time.

as HEAR, impurities have largely gone unobserved, and in many instances where non-GM oilseed rape volunteers emerge in a non-GM oilseed rape crop, the percentage impurity is unknown and is ignored. The potential of a crop variety to convey an impurity is not the same for all varieties but differs depending on factors such as the proportion of seeds that enter dormancy, the persistence of seeds in the soil and the relative pollen 'strength' of potential donor and recipient fields (for example caused by different proportions of male sterile plants). These factors may or may not differ between GM and non-GM varieties but should not differ because one is GM and the other not. They would differ in any case. 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.

**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 (eg, 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?**

**Dr. Nutti**

1181. Answer 104: As I pointed out before, 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.

**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?**

**Dr. Nutti**

1182. Answer 105: I am of the opinion 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.

1183. It is important to point out that usually the novel non-biotech products, such as plant products produced by selective breeding, cross-breeding and induced mutagenesis have not been subjected to any extensive safety assessment as the plants derived from the Recombinant-DNA technology have been.

1184. I also think that the methodologies which will be used for this monitoring need to be evaluated very carefully since it will be very difficult to monitor any effect that we are not aware of.

Therefore, I think this is one good point to be introduced in the agenda of the Codex Alimentarius Task Force on Foods Derived from Biotechnology, which will start working in September 2005.

**Dr. Andow**

Rationale for monitoring:

1185. There are some biotech products at issue in this dispute for which one committee or another has determined that there were no significant risks. I have taken issue with some of these determinations in my previous responses. 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.

1186. This disagreement provides the only rationale for monitoring potential adverse effects. If all parties agreed that there were no significant identified risks, there would be no need to consider monitoring for potential adverse effects.

1187. Unanticipated effects could arise from a transgene by any of the following mechanisms: new ORFs, insertional mutagenesis, post-transcriptional or post-translational processing, other pleiotropy or epistasis, and gene-by-environment interaction. New ORFs and insertional mutagenesis can be addressed through molecular characterization of all transgene loci. Post-transcriptional or post-translational processing can be addressed to the most part by molecular and biochemical analysis. Other pleiotropy or epistasis and gene-by-environment interaction cannot be addressed without extensive planting in the field.

1188. Under present technologies, transgenes can generate new ORFs and insertional mutagenesis. Post-transcriptional and post-translational processing may relate to the structure of the transgene and the nature of the gene product. Products novel to the plant probably require closer assessment. A gene product native to the recipient plant may be less subject to processing, except if it is expressed in new tissues. Novel products probably require closer assessment than native products for other pleiotropy or epistasis and gene-by-environment interaction.

1189. Monitoring can be used to look for unanticipated effects.

1190. A less thorough molecular and biochemical characterization of the transgene locus and transgene products provides increased potential for unanticipated effects.

1191. Monitoring may be partially substitutable for molecular and biochemical characterization.

1192. Monitoring may also substitute for identifying all possible effects, by covering various categories of unanticipated effects. If a thorough molecular analysis is conducted, it would seem important to ensure that monitoring for the remaining unanticipated effects is not too expensive.

Comparison with non-biotech varieties:

1193. For monitoring related to a specific potential adverse effect, this would have to relate to the specific genes/ traits in the transgenic crop. For monitoring for unanticipated effects, a more general approach is needed.

1194. First, I should make clear that some conventional non-transgenic plants have risks, some of which may justify monitoring (and management).

1195. For potential adverse effects, the reasoning I have used here implies that the potential adverse effect is not a potential adverse effect from a conventional non-transgenic plant. Otherwise there would be no disagreement as I have posited in paragraph 1185.

1196. For unanticipated effects, there is a quantitative difference in some of the concerns related to the process of transgenesis (paragraph 1162), and differences related to the novelty of the transgene product (paragraph 1188). Native biotech products may or may not be different from conventional breeding for unanticipated effects stemming from other pleiotropy or epistasis and gene-by-environment interaction.

### **Dr. Snow**

1197. My conclusion is that it does not seem logical to require monitoring if no risk has been identified (see Question 99). Also, both transgenic methods and other methods used crop breeding can result in unintended effects, and in my opinion there is no basis to assume that these unintended effects would be substantially greater in transgenic plants.

**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?**

### **Dr. Andow**

#### Rationale for management:

1198. There are some biotech products at issue in this dispute for which one committee or another has determined that there were no significant risks. I have taken issue with some of these determinations in my previous responses. 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.

1199. This disagreement provides the only rationale for risk management. If all parties agreed that there were no significant identified risks, there would be 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.

### **Dr. Squire**

1200. Provided no significant level or type of risk has been detected, then no particular change in practice above the highest recommended existing practice to ensure a high purity, should be needed. As in Q.103, however, if thresholds are imposed for whatever reason, 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. These practices are well appreciated – reducing volunteers to a minimum by appropriate soil cultivation after harvest, preventing volunteers from flowering in subsequent crops, leaving several years before growing the same type of crops again so that volunteer seed decays by natural means, leaving a separation strip between crops which is not harvested and beyond which gene flow is reduced to very low values, and not planting crops having reduced self-pollen in the vicinity.

1201. 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.

**Foods produced with biotech processing aids<sup>140</sup>, including yeasts, bacteria or enzymes that have been modified using recombinant DNA technology**

**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).**

**Dr. Nutti**

1202. Answer 107: According to the information before the Panel, and the existing bibliography in this field, my view is that the safety of the biotech products at issue in this dispute (including products subject to the member State safeguard measures) should be assessed according to the FAO/WHO Codex Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, in order to evaluate the potential risks to human, plant or animal health.

1203. For foods produced using biotech processing aids, including yeasts, bacteria or enzymes that have been modified using recombinant DNA technology, it is important to point out that the microorganisms which produces the processing aid was modified and NOT the processing aid itself. Therefore, I am of the opinion that 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.

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<sup>140</sup> "Processing aid" is defined by the *Codex Alimentarius* as "any substance or material, not including apparatus or utensils, and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or its ingredients, to fulfil a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product".

1204. It is important to recognize that in both cases the safety assessment should be carried out in order to evaluate the existence of any potential risks. If the intended use of the two different categories of products (foods and processing aids) is taken into account, the first point to be considered is that processing aids are used in very small amounts in food products, so the direct human or animal consumption of processing aids obtained through the recombinant DNA-technology will be much lower than foods derived from Recombinant-DNA plants. As far as further processing for consumption is concerned, most of the foods that are consumed nowadays is subjected to processing, so this point cannot be considered as a difference among the two product categories.

**Dr. Andow**

Kinds of risk:

1205. In general, foods produced with biotech processing aids are not expected to be able to self-reproduce in the environment. Thus the kinds of risk associated with these foods do not include any of the environmental risks associated with transgenic plants.

1206. Foods produced with biotech processing aids can have some non-target effects if the food is available in the environment. These effects are more similar to the effects of chemicals rather than the effects of living biological organisms.

Nature and magnitude of risk:

1207. I will not address human or animal health hazards. The following comment, however, has some implications for human and animal health hazards.

1208. As I have argued in question 9 and 102, in transgenic plants, unanticipated effects could arise from a transgene by any of the following mechanisms: new ORFs, insertional mutagenesis, post-transcriptional or post-translational processing, other pleiotropy or epistasis, and gene-by-environment interaction. For each of the potential sources of unanticipated effects, the likelihood of it occurring in a transgenic bacterium or yeast (that is typically used as a food processing aid) is much lower than in a transgenic plant. For transgenic bacteria and fungi (especially yeast), transgene insertion is more predictable and the probability of new ORFs is much lower. Transgenic bacteria and yeast are much easier to screen and eliminate insertional mutations. Moreover, there is considerably less post-transcriptional and post-translational processing of gene products in bacteria and yeast than in plants. Finally the genome size is smaller in bacteria and yeast and they are used in a much more restricted set of environments than plants, so risks associated with other pleiotropy and epistasis and gene by environment interaction are less.

**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 (eg, 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?**

**Dr. Nutti**

1209. Answer 108: As I pointed out before, the use of antibiotic resistance marker genes has been recognized as a tool that can be safely used, and that its use should be evaluated on a case by case basis. The same applies to processing aids, that is, the processing aids derived from Recombinant-

DNA microorganisms should be evaluated on a case by case basis. Therefore, the same safety assessment principle should be applied in both cases.

**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?**

**Dr. Nutti**

1210. Answer 109: 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.

1211. I also think that the methodologies which will be used for this monitoring need to be evaluated very carefully since it will be very difficult to monitor any effect that we are not aware of. Therefore, I think this is one good point to be introduced in the agenda of the Codex Alimentarius Task Force on Foods Derived from Biotechnology, which will start working in September 2005.

**Dr. Andow**

Monitoring and Mitigation:

1212. There are some biotech products at issue in this dispute for which one committee or another has determined that there were no significant risks. I have taken issue with some of these determinations in my previous responses. 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.

1213. This disagreement provides the only rationale for monitoring or mitigating potential adverse effects. If all parties agreed that there were no significant identified risks, there would be no need to consider monitoring for potential adverse effects.

1214. Unanticipated effects could arise from a transgene by any of the following mechanisms: new ORFs, insertional mutagenesis, post-transcriptional or post-translational processing, other pleiotropy or epistasis, and gene-by-environment interaction. New ORFs and insertional mutagenesis can be addressed through molecular characterization of all transgene loci. Post-transcriptional or post-translational processing can be addressed to the most part by molecular and biochemical analysis. Other pleiotropy or epistasis and gene-by-environment interaction cannot be addressed without extensive planting in the field.

1215. All of these possible sources of 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.

**Question 110: On the basis of the information before the Panel, is there any scientific evidence to support the hypothesis that animal feed made from biotechnology plants alters the composition of the food derived from animals consuming the feed?**

- (a) **If so, what is the likelihood that this event could lead to adverse effects on human or animal health? (see, *inter alia*, exhibit US-144)**
- (b) **What risk management options are available to mitigate any resulting risks and what is their efficacy?**

**Dr. Nutti**

1216. Answer 110: I am not aware of any scientific evidence that supports the hypothesis that animal feed made from biotechnology plants alters the composition of the food derived from animals consuming the feed. One of the studies provided at US-144 showed no difference in milk produced from lactating cows fed rations with genetically modified whole cottonseed to milk produced from cows fed non genetically modified but genetically similar cottonseed. The study showed that no milk samples were positive for transgenic or plant DNA fragments at the limits of detection for the assays following detailed data evaluation criteria. The DMI, milk yield, milk composition, body weight and body composition score did not differ among treatments. The study showed that cottonseed from genetically modified varieties used in these studies yielded similar performance in lactating dairy cows when compared to non transgenic control and reference cottonseed.

1217. The second study used dehulled soybean meal prepared from genetically modified, herbicide tolerant soybeans and near isogenic conventional soybeans in an experiment with growing-finishing pigs. The study concluded that rate and efficiency in weight gain, scanned backfat, longissimus area and calculated carcass lean percentage were not different for pigs fed diets containing conventional or Gm soybean meal. Composition of the meat and sensory scores were not influenced by diet. The results indicated that Roundup Ready soybean meal is essentially equivalent in composition and nutritional value to conventional soybean meal for growing-finishing pigs.

1218. These are not the only studies that had been carried out for the comparison of composition of the food derived from animals consuming GM grains, plants or meals. Dr. Marjorie Faust, from Iowa University has conducted this type of studies since 1997, with different crops and different animals, and up to now, the results showed no difference in the composition of the products or in the animal performance.<sup>141</sup>

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<sup>141</sup> References:

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**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?**

**Dr. Nutti**

1219. Answer 111: As I had pointed out in several answers, and based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 34 to 43, Assessment of Possible Toxicity, we have at paragraph 37 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. **My understanding is that if the toxicological assessment has presented safety studies on protein digestion in mammalian gastric and intestinal systems, acute gavage studies in mice, homology to known toxins and allergens, and exposure to human diet that has no indication of adverse effects, and the comparison of the GM and its conventional counterpart has been properly conducted and showed equivalence, there is generally no reason to expect any remaining risk.**

1220. The relevance of the end product use(s) is considered in the safety assessment, if we consider that we have the comparison not only between the GM grain or plant and its conventional counterpart, but we also have to compare the composition of products thereof, for instance in the case of soybeans the comparison has considered different processed products as: soy meal, defatted soy meal, toasted flour, non toasted flour, refined oil, isolated protein and extruded soy protein.

**Dr. Snow**

1221. In general, the scientific arguments in paragraphs 128-133 are valid, but the US does not acknowledge possible inherent shortcomings of small-scale, short-duration field tests. The US correctly states that: "Such tests are designed primarily to examine whether the bioengineered plant exhibits the intended characteristics." However, these tests rarely address environmental risks such as the potential consequences of interbreeding with wild or weedy relatives, as the US claims. In paragraph 131, the US states that "effects on other organisms in the ecosystem" are evaluated, including "indirect impacts, such as those on farming production methods". However, the methods for making these evaluations are not explained or cited.

1222. Here, I think it is important to point out that these same questions are central to many of the concerns that several EC Member States have raised. These are the types of questions that sometimes suggest the need for further research, which takes time, resources, and multidisciplinary collaborations. 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.

1223. The foreseen end product uses of the product are relevant to safety evaluations – this is a standard assumption in all risk assessments, so I am not sure what the point of this question is.

**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?**

**Dr. Nutti**

1224. Answer 112: As I had pointed out in several answers, and based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 34 to 43, Assessment of Possible Toxicity, we have at paragraph 37 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. My understanding is that if the toxicological assessment has conducted acute gavage studies in mice, homology to known toxins and allergens with no indication of adverse effects, chronic toxicity tests shall not be required.

**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?**

**Dr. Nutti**

1225. Answer 113: Based on the Codex Alimentarius Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003), paragraphs 53, my understanding is that additional animal feeding studies may be warranted for GM foods if changes in the bioavailability of the nutrients are expected or if the composition of the GM food is not comparable to conventional food. My understanding is that if the comparison of the composition of the GM product and its conventional counterpart has shown to be substantially equivalent, the 90 –day oral study and at least one more feeding study ( usually 48 day broiler chicken ) has provided information on the consumption of the whole food, the request for multiple whole food studies in different species does not have scientific support.

**Question 114: Please provide an assessment of the statements regarding small random DNA insertions and molecular characterization in paragraph 152 of the US Supplemental Rebuttal Submission.**

**No Answers.**

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