UNEP

CBD

CONVENTION ON BIOLOGICAL DIVERSITY

Distr.
GENERAL

UNEP/CBD/BS/RW-RA&RM/INF/2
14 August 2007

ORIGINAL: ENGLISH

AFRICAN REGIONAL WORKSHOP ON CAPACITY- BUILDING
AND EXCHANGE OF EXPERIENCES ON RISK ASSESSMENT
AND RISK MANAGEMENT OF LIVING MODIFIED ORGANISMS
Addis Ababa, 23-25 August 2007

AN OVERVIEW OF SUGGESTED ADDITIONS TO THE FORMAT FOR RISK ASSESSMENT SUMMARIES SUBMITTED TO THE BIOSAFETY CLEARING-HOUSE

Note by the Executive Secretary

1. The Executive Secretary is pleased circulate herewith, for the information of participants, a document submitted by the resource person for the workshop containing proposals for additional elements to the current format for risk assessment summaries submitted to the Biosafety Clearing-House in accordance with paragraph 3 (c) of Article 20 of the Protocol. The document has been prepared to assist in addressing some of the gaps identified in the information contained in risk assessment summaries submitted to the Biosafety Clearing-House. The document also includes an example from the Netherlands of a completed Risk Assessment Summary Format for transgenic maize with an insecticidal protein, a Bt toxin (1507 maize).

2. The document is intended to facilitate discussions at the workshop on the need to further develop the common format for presenting risk assessment summaries to the Biosafety Clearing-House and on additional core elements for the common format to be recommended for consideration by the Conference of the Parties serving as the meeting of the Parties to the Protocol at its fourth meeting.

3. The document is being circulated as it was received from the resource person.

OVERVIEW OF THE ADDITIONAL INFORMATION FOR THE BCH RISK ASSESSMENT SUMMARY FORMAT

Explanatory note:
The text is organized as follows:

Red text: the present BCH format for risk assessment summaries

- ***Open bullets:*** *proposed additional headings in the RA Summary Format*

Italicized *text describes proposals for additional information to be asked. This text would be part of the new format for risk assessment summaries.*

Rationales and **Notes** provide background information on why the additional information is asked, and comments.

General information

- **Country taking the decision or making the declaration:**

- **Title of risk assessment**

- **Competent National Authorities**

Specification of the methodology of Risk Assessment

- *Provide additional information on the methodology of environmental risk assessments (RAs) performed by this CA, on the scope and endpoints of RA in general, as well as the specific scope and endpoints of this RA if that is a subset of the general scope.*
- *Provide links to applicable legislation, guidance documents and other relevant documents.*

Rationale:
The scope and endpoints of a particular RA are an important indicator to determine whether the RA is interesting for a another case. For instance an RA that has environmental risks of import, but not cultivation, of an LMO as its endpoint has more limited applicability than an RA that does take into account cultivation.
Note: It could be useful for uniformity in the database, to present here a drop-down list of endpoints from which a choice can be made.

Electronic files
Provide links to electronic files of the text of the notification and related documents, if publicly available on the internet.
Note: These links may also be provided under 'Additional Information'. It would however appear to be useful to have at least links to the text of the notification presented here, if they are available.

LMO Information

- **Living Modified Organism**

Characteristics of the recipient organism

Provide information on the characteristics of the recipient organism that are relevant to the RA.
Rationale
The generally accepted methodology for an RA takes into consideration the characteristics of the recipient organism, of the introduced traits, the (expected) new traits of the LMO, and the (expected) interaction of the LMO with the receiving environment.
It appears that the question on the characteristics of the recipient organism has been left out in the summary. But it is important to know what information on the recipient organism has been used by the risk assessor to value the outcome of the RA.

Note: It may be useful to suggest the notifier to refer to relevant documentation on the recipient organism (if it is available), such as the OECD consensus document on this issue.

Characteristics of modification

- Vector characteristics (Annex III 9 (c))
- Insert or inserts (Annex III 9 (d))

Rationale for the following additions:
The terms 'vector characteristics' and 'insert' may be confusing. Therefore we propose to add the following 'points to consider' for providing information on the method of transformation, and on the characterization of the DNA that has been inserted into the genome of the recipient.

Information on the characteristics of the vector is useful together with information on the method of transformation, e.g. *A. tumefaciens* transformation. Therefore we propose to add:

Method of transformation
Describe the method of transformation and the vector/DNA sequences used in the transformation process

The 'insert' might mean: the gene cassette present in the vector, or: the DNA that actually has been inserted into the genome of the recipient. To clarify this, and in order to obtain the information on characterization that is needed for risk assessment, we propose to add:

Molecular characterization of DNA inserted into the genome of the recipient:

- ***Verification of data:***
  Which criteria were used to check the completeness and validity of the data supplied by the notifier.
- ***Characterization of insert: type and use of data provided by the notifier***
- ***Provide the type and use of data,*** *for instance: hybridization data, used for determining copy numbers and the over all structure of the insert; sequence data, used for detailed characterization.*
- ***Analysis of data***
  Provide an interpretation of the characterization data, in terms of genes and relevant ORFs that are expected to be expressed.
- ***Conclusion of the molecular characterization:***
  Provide the explicit conclusion drawn from the data, and the list of items stemming from the molecular characterization that are relevant for the RA.

Functional characterization of the coding sequences inserted into the genome of the recipient:

- ***Verification of data:***
  Which checks have been performed by the risk assessor of the completeness and validity of the data supplied by the notifier. Have any other sources of data been used?

- ***Function of gene products***
  What is the function of the genes and ORFs identified as relevant for the RA in the molecular characterization.
- ***Level of expression of expected gene products***
  Provide the level of expression in absolute terms and/or in relative terms, e.g. as percentage of total dry weight.
- ***Conclusion of the functional characterization***
  Provide the explicit conclusion drawn from the data, and the list of items stemming from the functional characterization that are relevant for the RA.

Conclusion of the molecular and functional characterization
Specification and rationale which items will be taken into account in further RA

Detection and identification of the LMO
Suggested detection and identification methods and their specificity, sensitivity and reliability (Annex III, paragraph 9(f))Intended use and receiving environment

- **Intended use of the LMO (Annex III 9 (g))**
  Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms

- **Receiving environment (Annex III 9 (h))**
  Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.
  Provide a general discussion on the expected impact of the intended use of the LMO on the receiving environment, and how this is taken into account within the scope of the RA.

Risk assessment summary

- **Summary of risk assessment**
  This field can be used ~~as an alternative to, or~~ in addition to, the six specific text boxes that follow and correspond to paragraphs 8a to 8f of Annex III of the Biosafety Protocol.
  Note: We would argue that this field can only be used to provide information *in addition to* the information provided under 'Further risk assessment summary information'. The detailed information is in fact what makes the summary useful.

Further risk assessment summary information
The fields below can be used as an alternative to, or in addition to, the risk assessment summary above.

In order to present a clear overview of the RA it is important that information is presented for each of the items (a) – (f) below. Specifically information is needed on:
- *what questions were taken into account and why or why not;*
- *how, on what grounds was it decided that the information provided is sufficient (e.g. 'this answers the questions of a worst case approach')*

- **(a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health. (Annex III 8 (a))**
  This step of the RA process is commonly called 'hazard identification'. The information should focus, next to the identification of a novel phenotype, on cause – effect relationship between the phenotype and the adverse environmental effects.

- **(b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism. (Annex III 8 (b))**

- **(c) An evaluation of the consequences should these adverse effects be realized.(Annex III 8(c))**

- **(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized.(Annex III 8 (d))**

- **(e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks.(Annex III 8 (e))**

- **(f) 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.(Annex III 8 (f))**

Detailed risk assessment information

- **Availability of detailed risk assessment information**
  Please indicate whether more details on the risk assessment are available and how they can be accessed.

Additional information

- **Any other relevant information**

- **Relevant documents (links to websites and files of documents may be added here)**

- **Notes**

EXAMPLE OF A COMPLETED RISK ASSESSMENT SUMMARY FORMAT: RISK ASSESSMENT REPORT FOR 1507 MAIZE

Note on the example:
We present here a summary of the risk assessment (RA) for a notification of commercial cultivation of the maize line 1507, in the way it would be performed by the Netherlands when it acts as 'lead Competent Authority (CA)' in the EFSA (the European Food and Feed safety Authority) procedure for market applications. Such a RA by the 'lead CA' covers only the environmental RA for cultivation of the LMO. It is subsequently used by EFSA as it formulates its opinion on the environmental as well as the food and feed safety evaluation.
This implies that the RA presented here only covers the environmental effects of cultivation of the LMO, i.e. it is an environmental risk assessment.

Explanatory note on the organization of the text:

Red text: the present BCH format for risk assessment summaries

Straight text: texts that we would expect to be presented in a RA summary, based on the current BCH format.

Text in italics: *additional information, as requested in the document 'Overview of additional information for the BCH RA summary'.*

General information

- **Country taking the decision or making the declaration:**
  The Netherlands

- **Title of risk assessment**
  Environmental Risk Assessment Report for 1507 maize

- **Competent National Authorities**
  Ministry of Environment
  Ms. Inge Van der Leij
  Directorate for Chemicals, Waste and Radiation Protection; Division for Radiation Protection, Nuclear and Biosafety/IPC 645
  Ministry of Environment
  NL-2500 GX
  The Hague
  The Netherlands, P.O.Box 30945
  Tel: +31 70 339 4893
  Fax: +31 70 339 1316
  Email: inge.vanderleij@minvrom.nl
  Url: http://www.vrom.nl/ggo-vergunningverlening

- ***Specification of the methodology of Risk Assessment***
  Applicable legislation:
  *The environmental RA for market applications is regulated by the EU Directive **2001/18/EC**, part C. The environmental RA methodology is explained in Annex II of this Directive; information requirements are specified in Annex III. Further guidance on environmental RA is provided in **2002/623/EC***
  The scope of RA under Directive 2001/18/EC is: environmental risk assessment, including human health (in the case of incidental exposure), but not food and feed safety (in the case of chronic exposure) assessment.

  Endpoints of this RA:
  This RA takes into account adverse effects to the environment, including effects on human and animal safety from incidental exposure in the field and handling during agronomic practice (e.g. harvesting). Also taken into account are effects of the application of the LMO on agronomic practice, from a general point of view of sustainability.
  Food / feed safety is not an endpoint in this RA; food / feed safety assessment is performed in a separate procedure.

- ***Notification, electronic files***
  The public information in the dossier of the notifier can be found on the internet: http://www.vrom.nl/ggo-vergunningverlening. Further information on the status of 1507 maize can be found at http://www.gmo-compass.org/eng/gmo/db/65.docu.html.*

* The history of the handling of notifications for 1507 maize is complicated. The Netherlands handled a notification for import for food and feed use, number C/NL/00/10. Spain handled the application for import, processing and planting under the number C/ES/01/01. The Netherlands has published public information in these notifications on the internet, in its database 'ggo-vergunningverlening'. On this page click the links: 'vergunningendatabase' (in the text), and 'De database zelf' (at the bottom of the page), set 'procedure' to 'Alle'; at 'dossiernummer' type 'C/NL/00/10'; set status to 'alle'; click 'zoek'. On the next page click 'C/NL/00/10' to get the first of five pages with lists of the electronically available material (this also shows the complexity of this type of dossiers). Choosing 'dossier nummer': 'C/ES/01/01' leads to information on the Spanish dossier, mainly the assessment report. The EFSA has handled the same notification, under the same number. The dossier will become available on EFSA-net, but only after an EU decision has been taken. At the end of the RA summary we add links to the assessment reports of the Netherlands and Spanish CAs and the EFSA Opinion that is available, on the use of 1507 maize as or in food.

LMO Information

- **Living modified organism**

The LMO is *Zea mays* (maize, corn), expressing CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide.

The UI of the LMO is DAS-Ø15Ø7-1

- ***Characteristics of the recipient organism***

For information on the recipient organism, maize, refer to the OECD 'Consensus Document on the Biology of Zea mays (Maize)'.
Based on this information and on general familiarity obtained in decades of cultivation of non-LMO maize, it has been concluded in previous risk assessments for LMO maize by this CA as well as by the EU, that the species is biologically contained within Europe. It depends on human intervention for survival. There are no wild populations of maize known in Europe. Maize has no sexually compatible wild relatives in Europe. Zea mays is able to hybridize with wild species of the genera Trypsacum and Zea; these species are limited in geographical area to Mexico and Guatemala. The dispersal of maize genetic material through hybridization is limited in Europe to dispersal to other cultivated maize.

Characteristics of modification

- **Vector characteristics (Annex III 9 (c))**
- **Insert or inserts (Annex III 9 (d))**

To obtain genetically modified maize line 1507, maize embryos were modified by particle acceleration with a gel-purified linear DNA fragment of 6235 base pairs (PHI8999A). The fragment was obtained from vector PHP8999 and contains the *cry1F* gene and the *pat* gene. The *cry1F* gene is regulated by the maize *ubiZM* 1(2) promoter and the ORF25PolyA terminator from *Agrobacterium tumefaciens*. The *pat* gene is regulated by the 35S CaMV promoter en 35S terminator from Cauliflower mosaic virus.
The fragment PHI8999A was demonstrated to be nearly completely present in line 1507. Further analysis revealed that on the 5' flanking side of the PHI8999A fragment in line 1507 another DNA sequence has been inserted, containing three ORF's: two ORF's corresponding to maize DNA and one ORF (ORF3) that consisted of part of the *cry1F* gene, the maize chloroplast *rpoC2* gene and the *ubiZM* promoter. ORF3 codes for a hypothetical protein of 250 amino acids.

Molecular characterization:

- ***Source and verification of data:***

All data on molecular characterization were supplied by the notifier.
They were screened for completeness and consistency, e.g. for proper controls in hybridization data, sequencing data stretching the entire insert, state-of-the-art application of bioinformatic analysis.
Hybridization data were presented with high quality images of the actual results of experiments.
No further checks have been performed.

- ***Transformation method:***

The notifier has indicated that maize embryos were modified by particle acceleration with a gel-purified linear DNA fragment containing the DNA sequences intended to be introduced into the LMO: the cry1F gene and the pat gene.

- ***Characterization of insert: type of data provided by the notifier:***

We verified that Southern hybridization data provided by the notifier showed the presence of one insert containing a single copy of the linear DNA fragment used for transformation, and an additional fragment at its 5' side.
The complete DNA sequence of the insert was provided by the notifier.

- ***Analysis of data:***

From the Southern hybridization data provided by the notifier we verified that the expected genes cry1F and pat and their regulatory sequences were present in line 1507.
We also verified that the Southern hybridization data showed the presence of at least part of the cry1F gene in the additional DNA fragment. Bioinformatic analysis performed by the notifier, on the sequence data

that he has provided, revealed that this additional cry1F sequence is part of an ORF (ORF3) that consists of part of the cry1F gene, the maize chloroplast rpoC2 and the ubiZM promoter. ORF3 codes for a hypothetical protein of 250 amino acids.
We verified that Southern hybridization data showed that no sequences of the backbone plasmid were present; in particular the nptII gene, coding for kanamycin resistance, that is present on the backbone plasmid, was shown to be absent in line 1507.

- ***Conclusion of the molecular characterization:***
  From the experimental data supplied by the applicant it was concluded that the molecular characterization of line 1507 was sufficient to substantiate the above-mentioned presence of the cry1F and pat gene and absence of the vector backbone sequences. The presence of ORF3 must also be taken into account in the functional characterization.

Functional chracterization

- ***Source and verification of data:***
  All information on functional characterization was supplied by the notifier. The data included unpublished results as well as references to peer reviewed scientific literature. Unpublished information was screened for consistency and conformity with scientific standards, e.g. use of state-of-the-art methods, and the inclusion of results of proper controls.
- ***Function of the expected gene products:***
  cry1F:
  The cry1F gene is a synthetic version of the truncated cry1F gene of B. thurigiensis subsp. Aizawi, optimalized for plant codon usage. The plant-produced CRY1F toxin is equivalent to the truncated form of the bacterial CRY1F toxin that is the active form of the bacterial toxin. The CRY1F toxin is effective against lepidopteran species, the maize borer pests Ostrinia nubilalis, Sesamia nonagrioides and Diatraea grandiosella, and Spodoptera frugiperda and Agrotis ipsilon.

  pat
  The pat gene is a synthetic version of the glufosinate ammonium tolerance gene (phosphinotricine acetyl tranferase) of Streptomyces viridochromogenes strain Tü494, optimized for plant codon usage
  The pat gene product inactivates the herbicidal activity of phosphinotrycin.

  For ORF3 no homology was detected for with known toxins or allergens, except for the partial homology with CRY1F.

- ***Level of expression of expected gene products***
  cry1F
  The CRY1F toxin, is expressed in all parts of the LMO. Expression is in the order of 0.5-1ng/µg total extractable protein. This is within the normal range of expression of Bt toxins in LMOs.

  pat
  Expression of PAT is only detectable in leaves, and is in the order of 0.05-0.1 ng/µg extractable protein.

  The level of expression of ORF3 was assessed by RNA assays, Northern blots and rtPCR. We verified that no expression products could be detected.

- ***Conclusion of the functional characterization***
  The presence of the cry1F and pat genes and the level of expression of their gene products and resulting phenotypes have been taken into account in the RA.

Conclusion of the molecular and functional characterization
Expression of the CRY1F toxin and the PAT protein ***has to be taken into consideration in the RA.***
The expression of the predicted gene product of ORF3 is at most very low. The gene product has no known function, so no phenotype can be predicted that would be based on expression of these gene products. In any case, the gene products have no homology with known toxins or allergens. On the basis of these considerations, the presence of ORF3 ***has not been taken into consideration in the rest of this RA****.*

Detection and identification of the LMO

- **Suggested detection and identification methods and their specificity, sensitivity and reliability (Annex III, paragraph 9(f))**
  The applicant has provided a PCR detection method that is specific for event 1507, as is obligatory under the Directive 2001/18/EC. The PCR detection method together with the corresponding reference material will be made available to the regulatory authority and the European Commission Joint Research Centre (JRC).

Intended use and receiving environment

- **Intended use of the LMO (Annex III 9 (g))**
  Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms
  1507 maize will be placed on the market in all Member States of the EU for all uses of conventional maize, including cultivation.

- **Receiving environment (Annex III 9 (h))**
  Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.
  The receiving environment is arable land in the EU that is also available for the cultivation of conventional maize varieties.

  This RA takes into account the adverse affects that cultivation of 1507 maize may have in the agronomic environment. There are no wild relatives of maize in any of the Member States of the EU, therefore the scope of the risk assessment is limited to the agronomic environment in the EU where maize is commercially cultivated.

Risk assessment summary

- **Summary of risk assessment**
  This field can be used as an alternative to, or in addition to, the six specific text boxes that follow and correspond to paragraphs 8a to 8f of Annex III of the Biosafety Protocol.
  The risk assessment summary is presented in detail in the section 'Further risk assessment summary information'.

Further risk assessment summary information

- **(a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health. (Annex III 8 (a))**

  Note: *The information presented in the following summary RA, may be presented in a more intuitive way, that fits better to the methodology prescribed in Annex II of 2001/18/EC. This way of presenting can be found in the Annex to this document.*

  The genotypes and resulting phenotypes taken into consideration are:
  - *cry1F*, resulting in resistance to maize borer pests, and potentially leading to harm to non-target organisms, including potential effects on human health;
  - *pat*, resulting in tolerance to glufosinate ammonium-based herbicides

  The aspects taken into account in order to evaluate the potential adverse effects on human and animal health and the environment are:
  - Capacity of survival, establishment and dissemination.
  - Potential for genetic transfer.
  - Genetic stability of the insert
  - Effects on target organisms.
  - Effects on other organisms.
  - Adverse effects to humans and animals (including toxic and allergenic effects)
  - Expected changes in agronomic practice due to use of the LMO
  These are the aspects that have to be taken into account according to Annex II of Directive 2001/18/EC.

  As to genetic stability, the Mendelian inheritance pattern of the traits was assessed by the notifier, together with the physical linkage of the target genes in resulting progeny. Southern blots and maintenance of the phenotype indicated genetic and phenotypic stability of the transgenic line and their progeny over several generations. No

instability of the DNA sequences flanking the insert was observed.
Conclusion: the issue of genetic stability is therefore not taken into consideration in the rest of the RA.

The other aspects are taken into account in hazard identification. The following hazard scenarios have been identified, and are thought to be exhaustive:

For CRY1F:
We have taken the following scenarios into consideration:
- *Capacity of survival, establishment and dissemination might be enhanced by the insect resistant phenotype, if insect damage is an important factor in controlling population size of insect sensitive maize.*
- *Potential for genetic transfer: outcrossing of insect resistance to wild relatives of maize would give rise to insect resistant populations of the wild relatives. These would have to be subjected to RA, similar to the RA of 1507 maize.*
- *Effects on target organisms: extensive exposure to CRY1F might give rise to CRY1F resistant populations of the target organisms, Ostrinia nubilalis and Sesamia nonagrioides.*
- *Effects on other organisms: exposure of non-target insects that are sensitive to CRY1F might endanger these insect populations.*
- *Disease to humans and animals (including toxic and allergenic effects): as CRY1F is a toxin, albeit with a very narrow specificity (i.e. a number of lepidopteran species), incidental ingestion or other ways of exposure of humans and other vertebrates to CRY1F toxin might lead to toxic effects. Exposure might also lead to allergenic effects in humans.*
- *Expected changes in agronomic practice due to use of the LMO: application of the LMO might reduce the use of other less specific pesticides. It should be noticed that effects on pesticide usage are not dealt with under 2001/18/EC or 1829/2003/EC.*
  Conclusion: this scenario is not considered further in the RA.

for PAT:
We have taken the following scenarios into consideration:
- *Capacity of survival, establishment and dissemination might be enhanced by the herbicide tolerant phenotype, but only under circumstances where this presents a selective advantage, i.e. in the agronomic environment, if the herbicide is applied.*
- *Potential for genetic transfer: outcrossing of herbicide tolerance to wild relatives of maize would give rise to herbicide tolerant populations of the wild relatives. These would have to be subjected to RA, similar to the RA of 1507 maize.*
- *Effects on target organisms: no effects have been identified of the pat gene product or of the herbicide resistant phenotype on target organisms. This aspect is not taken into account the rest of the RA.*
- *Effects on other organisms: no effects have been identified of the pat gene product or of the herbicide resistant phenotype on other organisms. This aspect is not taken into account in the rest of the RA.*
- *Disease to humans and animals (including toxic and allergenic effects): No toxic effects of the pat gene product have been identified. Exposure might also lead to allergenic effects in humans*
- *Expected changes in agronomic practice due to use of the LMO: it has been argued that due to the herbicide tolerant phenotype, the volume of herbicide use will be reduced because the herbicide tolerance enables farmers to use the herbicide more selectively, i.e. only when the need for treatment becomes apparent. This would also reduce the need for pre-emergence herbicide treatment. It should be noticed that effects on herbicide usage are not dealt with under 2001/18/EC or 1829/2003/EC.*
  Conclusion: this scenario is not considered further in the RA.

Some of the aspects, in particular the capacity for survival, establishment and dispersal, might also be influenced by changes in general agronomic traits of the LMO. Therefore the occurrence of such changes has also been taken into consideration.

Note: in the following sections (b) – (f) we present a further detailed elaboration of the line of reasoning started in the italic text of section (a). This text is presented as ‘additional information’, i.e. in italics, although one might argue that the BCH format already to some extent asks for this kind of detail.

- **(b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism.(Annex III 8 (b))**

cry1F:

- *Capacity of survival, establishment and dissemination:*
  Maize (Zea mays) is not an invasive crop due to factors specific for this species, such as lack of dormancy, causing seed germination to take place soon after shedding in the autumn, low survivability of the seed in the soil, and the sensitivity of the maize plantlets to frost. There is no indication that insect damage plays any role in controlling survival, establishment or dissemination. Maize production in the European Union requires the extensive application of agronomic practices. In any case, should volunteers occur in the cropping area, they are easily controlled.
  Conclusion: this issue is not considered further in the RA.

- *Potential for genetic transfer:*
  Maize does not have any wild relatives in the EU region, therefore genetic transfer is not an issue.
  Conclusion: this issue is not considered further in the RA.

  The conclusions of the two bullets above are based on general familiarity with the characteristics of maize; they are sufficiently known to allow the conclusion at the end of each bullet.

- *Effects on target organisms:*
  cases of development of resistance to CRY1 toxins have been reported for maize borer pests in the scientific literature, and therefore cannot be excluded for CRY1F and the maize borer pests considered in this RA.
  Conclusion: this issue <u>has to be considered further</u> in the RA.

- *Effects on other organisms:*
  The notifier has provided studies of the absence of toxicity of CRY1F to beneficial and non-target organisms: studies in non-target arthropods (Chrysoperla carnea, Hippodamia convergens, Danaus plexippus, Nasonia vitripennis, etc), bees (Apis mellifera), terrestrial organisms (Eisenia foetida, Folsomia candida), wildlife birds (Colinus virginianus) and aquatic organisms (Daphnia magna). These data are considered sufficient to conclude that the genetic modification does not cause any relevant potential change in the interactions between 1507 maize and non-target organisms.
  The issue of how to deal with potential non-target effects in LMOs expressing Bt toxins is still under consideration (e.g. Romeis et al.). We think that further development of methodologies to assess non-target effects is needed, but we think that the data supplied by the notifier provide sufficient ground to conclude that no unacceptable non-target effects will occur.
  Conclusion: this issue is not considered further in the RA

- *Adverse effects to humans and animals (including toxic and allergenic effects):*
  The RA only takes into account acute toxic and allergenic effects that may occur due to incidental exposure of humans to 1507 maize.
  The applicant provided information from studies, published in peer reviewed literature, of acute oral toxicity in mice with the CRY1F protein that have demonstrated its safety to human and animal health. No mortality, toxicity or adverse clinical signs were observed with the highest dose tested. i.e. 576 mg CRY1F (the equivalent of 576 gr maize total protein) per kg body weight. In studies performed by the notifier no toxicologically significant differences were observed in a thirteen week (90 days) oral toxicity study in rats regarding body weight, food consumption, food efficiency, clinical signs of toxicity, ophtalmological observations, neurological behaviour, clinical pathology, organ weights and gross or microscopic pathology.
  These studies by the notifier are considered sufficient to show that no acute toxic effects are to be expected after incidental exposure of humans to 1507 maize.
  Allergenicity assessment has been carried out by the notifier. These studies have consisted of
  - comparison of the amino acid sequence of CRY1F protein with the amino acid sequences of known allergens; conclusion: no homology found
  - degradation in simulated gastric fluid: degradation is rapid
  - expression level: relatively low

- post-translational glycosylation: is lacking
- thermal susceptibility: high.
These results, provided by the notifier, are taken as sufficient indication that the CRY1F protein do not pose a significant potential allergenic risk.
Conclusion: these issues are not considered further in the RA.

PAT:

- *Capacity of survival, establishment and dissemination:*
  Maize (Zea mays) is not an invasive crop due to factors specific for this species, such as lack of dormancy, low survivability of the seed in the soil, and the sensitivity of the maize plantlets to frost. Maize production in the European Union requires the extensive application of agronomic practices. In the unlikely case that any volunteers should occur in the cropping area, they are easily controlled by other non-selective herbicides and cultivation techniques.
  Conclusion: this issue is not considered further in the RA.

- *Potential for genetic transfer:*
  Maize does not have any wild relatives in the EU region, therefore genetic transfer is not an issue.
  Conclusion: this issue is not considered further in the RA.

- *Effects on target organisms: not applicable*
  Conclusion: this issue is not considered further in the RA.

- *Effects on other organisms:*
  The herbicide tolerance phenotype causes no effects on any organisms.
  Conclusion: this issue is not considered further in the RA.

- *Disease to humans and animals (including toxic and allergenic effects):*
  The PAT protein has a history of safe use in crops, both where toxicity as allergenicity is concerned.
  Conclusion: this issue is not considered further in the RA.

  These conclusions on the likelihood of adverse effects of PAT to occur are based on our experience with LMOs expressing the PAT protein, and the absence of effects of PAT in the history of use of the pat gene in LMOs; we consider the history of use as sufficiently extensive to allow the conclusion at the end of each bullet to be drawn, that the issues are not considered further in the RA.

Changes in general agronomic traits of the LMO:

- *The notifier has provided information showing that the LMO shows no changes in reproductive morphology and therefore no difference in establishment, vigour and competitivity is expected in 1507 maize with respect to other commercially available varieties. Except for the resistance against certain lepidopteran insects and tolerance to glufosinate-ammonium herbicide, the 1507 maize line does not show any differences in survival with respect to non-modified maize.*
  We take this as a sufficient indication that the general agronomic traits of 1507 maize are sufficiently similar to those of non-modified maize that no adverse environmental effects are to be expected in this context.
  Conclusion: this issue is not considered further in the RA.

Conclusion:
Based on these considerations, there is one remaining item that has to be considered in the rest of the RA: ***development of resistance to CRY1F toxin in maize borer pests, specifically Ostrinia nubilalis and Sesamia nonagrioides****.*

- **(c) An evaluation of the consequences should these adverse effects be realized.(Annex III 8(c))**

We consider that the following considerations are applicable to all cases of potential development of resistance to CRY1 toxins in maize borer pests.
Should target organisms develop resistance to CRY1F, the toxin would lose its usefulness as a pesticide, but only for these organisms.

This would not only apply to applications of cry1F as transgene in LMOs, but also to the use of the CRY1F protein, e.g. as sprayed pesticide.
The target organisms, maize borers, are specific for maize, and are very hard to control by sprayed pesticides due to their niche: inside the stalk of the plant.
The consequence of resistance development would be reversion to the situation before CRY1F maize was available.
In that case, insect control could be exerted through the application of other cry genes, or genes coding for other proteins that are harmful to insects.

- **(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized.(Annex III 8 (d))**

We consider that there is a possibility that resistance against CRY1F develops in the target organisms, maize borer pests.

- **(e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks.(Annex III 8 (e))**

Based on paragraph (d) we conclude that the notifier should develop an insect resistance management plan (IRM) to deal with resistance development.
The Monitoring Plan supplied by the notifier proposes an insect resistance management plan (IRM) for early detection of the potential development of resistance to the Bt toxin in the borer populations. For this purpose, sampling and analysis will be carried out in a selection of representative areas with an elevated level of maize borer pests (Ostrinia nubilalis and Sesamia nonagrioides).
Conclusion: we recommend to effectuate the monitoring plan when 1507 maize is cultivated.

- **(f) 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.(Annex III 8 (f))**

Detailed risk assessment information

- **Availability of detailed risk assessment information**
  Please indicate whether more details on the risk assessment are available and how they can be accessed.

Additional information

- **Any other relevant information**

- **Relevant documents (links to websites and files of documents may be added here)**

Netherlands assessment report:
http://213.154.234.72/gits/(bshi0brkml3u0n55v2pbgoik)/Document.aspx?ID_DOCUMENT=37191
Spanish assessment report:
http://213.154.234.72/gits/(bshi0brkml3u0n55v2pbgoik)/Document.aspx?ID_DOCUMENT=37545
EFSA opinion on the placing on the market as food:
http://www.gmo-compass.org/pdf/regulation/maize/1507_maize_opinion_food.pdf

- **Notes**

Annex: alternative way for presenting the information on the RA
In this example the information on the RA is presented in a way that is felt by some to be more intuitive. However, the presentation follows the format of the BCH less strictly. This could be a disadvantage, as using a less strict format might lead to less harmonized presentation of the RA summary.

The genotypes and resulting phenotypes taken into consideration are:
- *cry1F*, resulting in resistance to maize borer pests, and potentially leading to harm to non-target organisms, including potential effects on human health;
- *pat*, resulting in tolerance to glufosinate ammonium-based herbicides

The aspects taken into account in order to evaluate the potential adverse effects on human and animal health and the environment are:
- *Capacity of survival, establishment and dissemination.*
- *Potential for genetic transfer.*
- *Genetic stability of the insert*
- *Effects on target organisms.*
- *Effects on other organisms.*
- *Adverse effects to humans and animals (including toxic and allergenic effects)*
- *Expected changes in agronomic practice due to use of the LMO*

These are the aspects that have to be taken into account according to Annex II of Directive 2001/18/EC.

1. Capacity of survival, establishment and dissemination
(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects
For CRY1F:
- *Capacity of survival, establishment and dissemination might be enhanced by the insect resistant phenotype, if insect damage is an important factor in controlling population size of insect sensitive maize.*

For PAT:
- *Capacity of survival, establishment and dissemination might be enhanced by the herbicide tolerant phenotype, but only under circumstances where this presents a selective advantage, i.e. in the agronomic environment, if the herbicide is applied.*

(b) Likelihood of these adverse effects being realized
Maize (Zea mays) is not an invasive crop due to factors specific for this species, such as lack of dormancy, causing seed germination to take place soon after shedding in the autumn, low survivability of the seed in the soil, and the sensitivity of the maize plantlets to frost.

For CRY1F:
– *There is no indication that insect damage plays any role in controlling survival, establishment or dissemination. Maize production in the European Union requires the extensive application of agronomic practices. In any case, should volunteers occur in the cropping area, they are easily controlled.*
This issue has not been considered further in the RA.

For PAT:
– *Maize production in the European Union requires the extensive application of agronomic practices. In the unlikely case that any volunteers should occur in the cropping area, they are easily controlled by other non-selective herbicides and cultivation techniques.*
This issue has not been considered further in the RA.

2. Potential for genetic transfer
(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects
For CRY1F:
- *Outcrossing of insect resistance to wild relatives of maize would give rise to insect resistant populations of the wild relatives. These would have to be subjected to RA, similar to the RA of 1507 maize.*

For PAT:
- *Outcrossing of herbicide tolerance to wild relatives of maize would give rise to herbicide tolerant populations of the wild relatives. These would have to be subjected to RA, similar to the RA of 1507 maize.*

(b) Likelihood of these adverse effects being realized
For CRY1F and for PAT:

– *Maize does not have any wild relatives in the EU region, therefore genetic transfer is not an issue.*
This issue has not been considered further in the RA.

3. Genetic stability of the insert

As to genetic stability, the Mendelian inheritance pattern of the traits was assessed by the notifier, together with the physical linkage of the target genes in resulting progeny. Southern blots and maintenance of the phenotype indicated genetic and phenotypic stability of the transgenic line and their progeny over several generations. No instability of the DNA sequences flanking the insert was observed.
This issue has not been considered further in the RA.

4. Effects on target organisms

(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects

For CRY1F:

- *Extensive exposure to CRY1F might give rise to CRY1F resistant populations of the target organisms, Ostrinia nubilalis and Sesamia nonagrioides.*

For PAT:

– *No effects have been identified of the pat gene product or of the herbicide resistant phenotype on target organisms.*

This issue has not been considered further in the RA.

(b) Likelihood of these adverse effects being realized

For CRY1F:

– *Cases of development of resistance to CRY1 toxins have been reported in the scientific literature for maize borer pests, and therefore cannot be excluded for CRY1F and the maize borer pests considered in this RA.*

(c) An evaluation of the consequences should these adverse effects be realized

For CRY1F:

– *The following considerations are applicable to all cases of potential development of resistance to CRY1 toxins in maize borer pests.*
Should target organisms develop resistance to CRY1F, the toxin would lose its usefulness as a pesticide, but only for these organisms.
This would not only apply to applications of cry1F as transgene in LMOs, but also to the use of the CRY1F protein, e.g. as sprayed pesticide.
The target organisms, maize borers, are specific for maize, and are very hard to control by sprayed pesticides due to their niche: inside the stalk of the plant.
The consequence of resistance development would be reversion to the situation before the situation before CRY1F maize was available.
In that case, insect control could be exerted through the application of other cry genes, or genes coding for other proteins that are harmful to insects.

(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized.

For CRY1F:

– *There is a possibility that resistance against CRY1F develops in the target organisms, maize borer pests. The notifier should develop an insect resistance management plan (IRM) to deal with resistance development*

(e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks

For CRY1F:

– *The Monitoring Plan supplied by the notifier proposes an insect resistance management plan (IRM) for early detection of the potential development of resistance to the Bt toxin in the borer populations. For this purpose, sampling and analysis will be carried out in a selection of representative areas with an elevated level of maize borer pests (Ostrinia nubilalis and Sesamia nonagrioides).*
This RA recommends to effectuate the monitoring plan when 1507 maize is cultivated.

5. Effects on other organisms

(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects

For CRY1F:

– *Exposure of non-target insects that are sensitive to CRY1F might endanger these insect populations.*

For PAT:

- *No effects have been identified of the pat gene product or of the herbicide resistant phenotype on other organisms.*

This issue has not been considered further in the RA.

(b) Likelihood of these adverse effects being realized

For CRY1F:

- *The notifier has provided studies of the absence of toxicity of CRY1F to beneficial and non-target organisms: studies in non-target arthropods (Chrysoperla carnea, Hippodamia convergens, Danaus plexippus, Nasonia vitripennis, etc), bees (Apis mellifera), terrestrial organisms (Eisenia foetida, Folsomia candida), wildlife birds (Colinus virginianus) and aquatic organisms (Daphnia magna). These data are considered sufficient to conclude that the genetic modification does not cause any relevant potential change in the interactions between 1507 maize and non-target organisms.*
  The issue of how to deal with potential non-target effects in LMOs expressing Bt toxins is still under consideration (e.g.Romeis et al.). We think that further development of methodologies to assess non-target effects is needed, but we think that the data supplied by the notifier provide sufficient ground to conclude that no unacceptable non-target effects will occur.
  This issue has not been considered further in the RA.

6. Adverse effects to humans and animals (including toxic and allergenic effects)

(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects

For CRY1F:

- *As CRY1F is a toxin, albeit with a very narrow specificity (i.e. a number of lepidopteran species), incidental ingestion or other ways of exposure of humans and other vertebrates to CRY1F toxin might lead to toxic effects. Exposure might also lead to allergenic effects in humans.*

For PAT:

- *No toxic effects of the pat gene product have been identified. Exposure might also lead to allergenic effects in humans*

(b) Likelihood of these adverse effects being realized

For CRY1F:

The RA only takes into account acute toxic and allergenic effects that may occur due to incidental exposure of humans to 1507 maize.

The applicant provided information from studies, published in peer reviewed literature, of acute oral toxicity in mice with the CRY1F protein that have demonstrated its safety to human and animal health. No mortality, toxicity or adverse clinical signs were observed with the highest dose tested. i.e. 576 mg CRY1F per kg body weight. In studies performed by the notifier no toxicologically significant differences were observed in a thirteen week (90 days) oral toxicity study in rats regarding body weight, food consumption, food efficiency, clinical signs of toxicity, ophtalmological observations, neurological behaviour, clinical pathology, organ weights and gross or microscopic pathology.

These studies are sufficient to show that no acute toxic effects are to be expected after incidental exposure of humans to 1507 maize.

Allergenicity assessment has been carried out by the notifier. These studies have consisted of the comparison of the amino acid sequences of the CRY1F and proteins with those of the known allergens, rapid degradation in simulated gastric fluid, relatively low expression level, lack of post-translational glycosylation and thermal susceptibility which confirm that the CRY1F proteins do not pose any significant potential allergenic risk.

These issues have not been considered further in the RA.

For PAT:

- *The PAT protein has a history of safe use in crops, both where toxicity as allergenicity is concerned.*
  This issue has not been considered further in the RA.

7. Expected changes in agronomic practice due to use of the LMO

(a) An identification of any novel genotypic and phenotypic characteristics that may have adverse effects

For CRY1F:

Application of the LMO might reduce the use of other less specific pesticides. It should be noticed that effects on pesticide usage are not dealt with under 2001/18/EC or 1829/2003/EC.

This issue has therefore not been considered further in the RA

For PAT:

- *Capacity of survival, establishment and dissemination might be enhanced by the herbicide tolerant phenotype, but only under circumstances where this presents a selective advantage, i.e. in the agronomic environment, if the herbicide is applied.*

(b) Likelihood of these adverse effects being realized

Maize (Zea mays) is not an invasive crop due to factors specific for this species, such as lack of dormancy, causing seed germination to take place soon after shedding in the autumn, low survivability of the seed in the soil, and the sensitivity of the maize plantlets to frost.

For CRY1F:

There is no indication that insect damage plays any role in controlling survival, establishment or dissemination. Maize production in the European Union requires the extensive application of agronomic practices. In any case, should volunteers occur in the cropping area, they are easily controlled.

This issue has not been considered further in the RA.

For PAT:

It has been argued that due to the herbicide tolerant phenotype, the volume of herbicide use will be reduced because the herbicide tolerance enables farmers to use the herbicide more selectively, i.e. only when the need for treatment becomes apparent. This would also reduce the need for pre-emergence herbicide treatment. It should be noticed that effects on herbicide usage are not dealt with under 2001/18/EC or 1829/2003/EC.

This issue has therefore not been considered further in the RA

Over all conclusion of the RA:

It is recommended to effectuate the monitoring plan provided by the notifier when 1507 maize is cultivated.
