Testing of the Roadmap, using a case-study of an LMO intended for release into the environment

This testing was carried out by the Chair of the AHTEG Sub-working Group (SWG) on the Roadmap for Risk Assessment in an attempt to enrich the discussions in the Open-ended Online Expert Forum and within the AHTEG SWG. The views expressed here are solely those of the SWG Chair based on a testing done using a case-study side-by-side the draft Roadmap and do not necessarily reflect those of other AHTEG members.

One of the important questions in the testing of the draft Roadmap is: *"Does the Roadmap help in the performance of an actual environmental risk assessment for the release of an LMO?"* To answer this question, an actual case-study of an LMO to be released into the environment was used in a testing ('Pre-market environmental risk assessment of transgenic plants: a case-study approach utilizing MON 15985 cotton', available as background document at http://bch.cbd.int/onlineconferences/roadmap3_ra.shtml). This case-study was kindly made available by Agbios (www.agbios.com), and is based on a dossier prepared by the Monsanto Company. The aim of such a test is to find out whether there are points where the Roadmap may be improved. Although while doing the test we will of course also find points where the case-study might be improved, it is not the purpose of the test to assess the case-study, or to perform a risk assessment of the specific case.

MAJOR COMMENTS:

Line references are made to the Draft Roadmap of 15 January, version 2.

Going through the case-study and the Roadmap, I felt that the Roadmap is helpful in pointing out many points that should be considered, but it utility could be increased if it would also point out more of the perspective why the various points should be considered, and how the different points to consider depend on each other. One example is mentioned below, in my general comments to step 4, and in other places relating to this same point, but there will be other places in the text where this could be done.

Step 1, … identification of any novel genotypic and phenotypic characteristics associated with the living modified organism … (from line 147 on).
In the case-study, a major adverse effect is the influence of the Cry gene products on non-target organisms in the environment. The questions in step 1 would not automatically lead to the development of this hazard scenario. In several places where interaction with the environment is mentioned, we might want to have a more detailed description of what types of scenario's for adverse effects may be envisaged.
The concept of hazard scenario, or any other concept of how to arrive at adverse effects from the characterization that is done in this step, is lacking. It would be very helpful if more attention is given to this issue.

Step 2, An evaluation of the likelihood of adverse effects being realized … (from line 208 on)
It is difficult to see the relationship between the points to consider in step 2, and the adverse effects identified in step 1.
In the case-study, several questions could be formulated, based on non target organisms that are expected to be present in the environment and what is the chance of them being exposed to the Cry gene products.

Step 3, An evaluation of the consequences should these adverse effects be realized (from line 241 on)
The concept of a 'baseline', that is crucial in the comparative approach of risk assessment, appears here for the first time. It might need to be explained, and maybe it should appear earlier, where the comparative approach is discussed.

Step 4, An estimation of the overall risk posed by the living modified organism … (from line 261 on)

The concepts of cumulative, synergistic, combinatorial effects presuppose knowledge about the physiological roles of the transgene products. In my detailed comments to step 1 (line 105-110 below, in this document) I am further elaborating on this.

DETAILED COMMENTS:

Below are detailed comments on specific parts of the draft Roadmap. Texts from the Roadmap are between quotes (“”), my own comments are shaded in gray.

Context and scoping of the risk assessment (line 109)

“The context and scope of risk assessment as laid down in existing policies and strategies, based on for instance regulations and international obligations of the Party involved …” (lines 113-114)

I have done this test from my perspective, as a regulator in the Netherlands, under the European Directive 2001/18/EC. In our practice this Directive sufficiently clear to as to context and scope of the risk assessment, i.e. the protection of human health and the environment from risks posed by the environmental release of LMOs.

“Framing the risk assessment process, taking into account the expected (potential) conditions of handling and use of the LMO, taking into account customary practices and habits that could affect the protection goals or end-points; identification of relevant questions to be asked for that purpose.” (lines 120-123)

It is not immediately clear exactly what the purpose of the release, described in the case-study, is. In an actual case of risk assessment this should be clear. The paragraph in the section Context and scoping of the risk assessment is useful to remind me to ask the applicant for a complete answer to the questions asked here, taking into account the points to consider.

“Experience and history of use of the non-modified recipient, taking also into account its ecological function” (lines 132-133)

This coincides with the point to consider (a) under Step 1, lines 164-168 and looks like a duplication. This point could be omitted here, and the text ‘taking also into account its ecological function’ could be added to the point to consider (a).

Step 1: … identification of any novel genotypic and phenotypic characteristics associated with the living modified organism … (line 147)

Points to consider regarding the characterization of the LMO (line 163)

“(a) Characteristics of the non-modified recipient (e.g. its biological characteristics, with particular attention to characteristics that, if changed, or interacting with other changed genes, could change the interaction of the non-modified recipient with the environment in a way that could cause adverse effects; its taxonomic relationships, its origin, centers of origin and centers of genetic diversity);” (lines 164-168)

The case-study provides an extensive description of the recipient species (paragraph 2A, pages 8-16. However, I think it would be useful provide a link here to other available documents on the biology of commonly used recipients, e.g. the OECD consensus documents; in this case. the OECD Consensus document on the biology of cotton (Gossypium spp.) would come in useful. I would also refer to the OECD document Points to consider for consensus documents on the biology of cultivated plants; this document presents an overview of such points to consider in the biology of a recipient, in the context of environmental risk assessment of LMOs. This document could be used to check the

completeness of the information that the regulator has available when performing the risk assessment.

"(b) Relevant characteristics of the genes that have been inserted into the LMO (e.g. functions of the gene product in the donor organism, with particular attention to characteristics that, when transferred to the recipient, could cause adverse effects);" (lines 169-171)

The information that is needed here may be described more precisely. Paragraph 2B in the case-study (pages 16-7) provides information on the donor organism, but with emphasis on the relevant gene products. What is also needed here is information on the *physiological role* that the gene product has in the donor organism, as well as in the recipient. All this is then an important trigger for the question about 'characteristics that, when transferred to the recipient, could cause adverse effects'.

"(c, *first part*) Molecular characteristics of the LMO related to the modification (e.g. transformation method, characteristics of the vector if and as far as it is present in the LMO, including its identity, source/origin and host range; characteristics of the insert(s), including gene products, expression level, function, its insertion site in the genome, stability or integrity) that are related to potential adverse effects. Availability and relevance of this information may vary according to the type of application, particularly at the stage of field releases*." (lines 172-177)

This part of paragraph (c) is clear and useful. It helps to ascertain that most of the necessary information is presented in paragraph 3A, pages 20-40 in the case-study.
It seems to me that this first part of (c) makes sense as a stand alone paragraph, where the molecular characterization concerns the changes made in the genome of the LMO. This should be specified here, because paragraph (c), second part, and paragraph (d) also (but not only) concern molecular characterization, but of expressed products.

"(c, *second part*) Characteristics related to adverse effects may also result from altered expression levels of endogenous genes due to effects of a transgene (e.g. due to insertional disruption of a gene or to regulatory effects) and combinatorial/synergistic effects of the transgene product with endogenous genes or products of other transgenes present in the LMO." (lines 178-181)

The second part of paragraph (c) makes sense on its own; the first part should be reworded to: 'Characteristics resulting from altered expression of …'

"(d) Identification of genotypic and phenotypic, biological changes in the LMO, either intended or unintended, in comparison with the non-modified recipient, considering those changes that could cause adverse effects;" (lines 182-184)

The points to consider in (c, second part) and in (d) concern the information that is presented in paragraphs 3B and 3C of the case-study (pages 40- 54). The issue of protein expression levels in different plant tissues (paragraph 3B-1, starting page 40) is not dealt with in paragraphs (c, second part) and (d), or elsewhere in step 1.
I think this issue should be taken into consideration in step 1, and it may be taken into consideration here, or as a separate item under the heading Points to consider regarding the potential adverse effects resulting from the interaction between the LMO and the receiving environment (Lines 191-192).
The case-study presents a phenotypic characterization of the LMO in paragraph 3.C of the case-study (pages 50-4). Of the characters referred to here, pest and disease susceptibility (pages 52-3) might be considered for specific mention in the Roadmap.

General comment: The case-study presents information on the characterization of the Cry2ab protein, and refers to similar data for the other expressed proteins, in paragraph 3.B-2, pages 45-50. This type of information is not mentioned specifically in the Roadmap, neither in step 1

nor elsewhere. It should be considered whether a question for this type of information should be added.

Step 2: An evaluation of the likelihood of adverse effects being realized … (line 208)

Points to consider

"(c) Levels of expression in the LMO and persistence and accumulation in the environment, e.g. in the food chain, of potentially harmful substances newly produced by the LMO, e.g. insecticidal proteins;" (lines 231-233)

Here again the issue of differential expression in different parts of the LMO is very important and should be added.

"(d) Available information on the location of the release (in case of confined releases, e.g. maps of release site*, biogeographical information), including information on the sexually compatible species, e.g. whether it is co-located with the LMO, and whether flowering occurs at the same time;" (lines 234-237)

There should be a clearer relation to the attributes of the receiving environment, that have been treated in paragraph (e) in step 1, lines 186-188.

Step 4, An estimation of the overall risk posed by the living modified organism … (line 261)

"c) Potential cumulative adverse effects due to the presence of multiple LMOs in the receiving environment, and synergistic/combinatorial potential adverse effects due to the presence of multiple transgenes or DNA sequences in the LMO and traits that may interact." (lines 280-282)

Cumulative effects are not taken into consideration in the case-study; I think that the presence of two different Cry gene products would call for this: how are they interacting? The terms cumulative, synergistic, combinatorial may have to be explained here. As I have already indicated in my general comments to this step, this point to consider presupposes knowledge about interactions of the transgene products at the physiological level, and we will have to pay more attention to that in other points to consider in other steps.

The term 'DNA sequences' is not clear here.