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AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT UNDER THE CARTAGENA PROTOCOL ON BIOSAFETY
Second meeting
Ljubljana, 19-23 April 2010

SUBMISSIONS ON THE IDENTIFICATION OF LIVING MODIFIED ORGANISMS OR SPECIFIC TRAITS THAT MAY HAVE ADVERSE EFFECTS ON THE CONSERVATION AND SUSTAINABLE USE OF BIOLOGICAL DIVERSITY, TAKING ALSO INTO ACCOUNT RISKS TO HUMAN HEALTH

INTRODUCTION

1. At its fourth meeting, the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP) established an Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management.[1]

2. According to its terms of reference as set out by the Parties, the AHTEG shall, at its second meeting, among other things, consider possible modalities for cooperation in identifying living modified organisms or specific traits that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

3. To assist the AHTEG in its deliberations, the COP-MOP requested Parties and invited other Governments and relevant organizations to submit scientifically sound information available at that time, on the identification of living modified organisms (LMOs) or specific traits that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

4. The COP-MOP also requested the Executive Secretary to compile the information received and to prepare a synthesis report for consideration by the AHTEG and the Parties.

5. In light of the above, the Secretariat sent out a notification to Parties, other Governments and relevant organizations on 28 May 2009.[2]

6. Six Parties (Burkina Faso, Colombia, European Commission, Mexico, Norway and United Arab Emirates), two non-Party countries (Australia, United States of America) and two organizations (Global Industry Coalition and Public Research and Regulation Initiative) have submitted their views on this issue as of 2 November 2009.

7. Some submissions included recommendations to the AHTEG while others had a list of scientific publications.

8. A compilation of the full submissions is annexed hereto. Submissions made in a language other than English were translated into English by the Secretariat. These translations are also annexed hereto.

[1] Decision BS-IV/11.

[2] Notification SCBD/BS/MPDM/jh/67587 (2009-056).

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E. NORWAY

Attachment 1

Response to the call from the CBD secretariat for "***submission of scientifically sound information regarding the identification of living modified organisms or specific traits that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health*".**

Introduction

Beforehand, there are a number of important considerations with respect to the scientific appraisal that are not only of value to risk assessors, but risk managers, when reviewing this information that we wish to make note of:

First, we wish to note to the CBD secretariat that it would also be useful to also request scientifically sound information that document not only adverse effects, but evidence of safety (as opposed to evidence no effects) for biodiversity and human health.

Second, it is important to acknowledge there are broad uncertainties surrounding the current scientific knowledge on the impacts of novel biologics into complex environments. This includes appraising the relevance empirical data collected within specific time and/or spatial scales under investigation, and especially within particular ecological or management contexts. Further, it must also be kept in mind the difficulty in extrapolation of small-scale experiments, or those using small sample sizes, which often can detect only large differences or effects, to real-world effects. In order to achieve sufficient statistical power, studies utilizing small sample sizes must accept higher levels of Type II error or "false negatives" that would miss effects that may indeed in reality be occurring within the scientific observation.

Clearly, more intensive empirical studies are needed to ascertain the likelihood of field-level impacts to biodiversity and human health. As widely agreed, the case by case approach can best inform what scientific aspects will be important and relevant parameters for the proposed site and conditions of investigation. In sum, the emergent uncertainties should not be equated with risk, but rather incorporated systematically into any risk characterization. That is, the science evidence may or may not be informative under certain scenarios or environments, but can, and should, inform and inspire certain scientific considerations or needed lines of biosafety investigation specific contexts. This kind of scientific information becomes particularly valuable as possible "early warnings", as without such data there exists no basis for opening potentially critical modes inquiry would otherwise be left unexamined, leading to insufficient protection of environmental and human/animal health. This is especially important where a precautionary approach is the desired norm, as stated in the Cartagena Protocol on Biosafety Article 1, which states its objective to be "[I]n accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development".

Thirdly, it should be noted that the request for scientifically sound information also should also follow with a scientifically sound and logical inference when interpreting this information. For example, a common logical fallacy in the interpretation of risk data is

that absence of evidence of harm is the same as evidence of absence of harm. More explicitly, the absence of observable effects *should not be interpreted as evidence safety for any particular effect*. The committees and working groups utilizing this information should not lose sight of this basic logic when drawing conclusions, especially from risk relevant scientific evidence derived from statistical hypothesis testing.

Lastly, and with the above in mind, we wish to call attention to a recent investigative report that appeared in *Nature* magazine (Waltz, September 3, 2009) that document *ad hominem* attacks and other threats towards scientists who have published empirical evidence of potential adverse effects of LMOs. The political fallout from such public controversy creates a kind of scientific silence, where biosafety investigators may fear retribution for merely publishing their experimental work. As one prominent scientist interviewed stated:

"When scientists are even afraid to ask the questions…that's a serious impediment to our progress" (Ibid., 32).

The main point we wish to highlight, is that these troubling developments in the discourse over LMOs likely have led, and will continue to lead to situations where the *adverse effects of LMOs are likely to be under reported, and under investigated.*

Given the often political nature of the scientific debates surrounding the vital issue of food production, many of the studies mentioned in this report are not without their critics. Nevertheless, much of the evidence give compelling insights into the dynamics of novel biologics into complex ecosystems and the difficulty in establishing safety of use of modern biotechnologies in agriculture, medicine, and animal husbandry. Clearly, further research needed to make informed decisions and conclusions. While appropriate policies regarding LMOs are not limited only to scientific considerations, science will play an important role in appraising potential risks.

With respect to scientific information, we wish to submit the following requested scientific information on the two classes of potential effects (A) unintended effects on biodiversity, which includes direct and indirect effects, and (B) unintended effects on human and animal health. Both groupings can be further categorized as direct and indirect effects. Please refer to the end of this report where all scientific studies and reports under discussion are cited.

A. Scientific information on LMOs or traits that "may have adverse effects on the conservation and sustainable use of biological diversity" including direct and indirect effects.

A1.1: Unintended direct adverse effects of Bacillus thuringiensis (Bt) Cry endotoxins on biological diversity, both lethal and sub-lethal, including but not limited to insects, aquatic life, soil microbes, and their food web dynamics

In two meta-analyses of published studies on non-target effects of Bt proteins in insects, Lovei and Arpaia (2005) document that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins. A review by Hilbeck and Schmidt (2006) on all Bt-plants found 50% of studies documenting negative effects on tested invertebrates.

Another quantitative review by Marvier et al, (2007) suggested a reduction in non-target biodiversity in GM in some classes of invertebrates (Bt) cotton fields vs. non-pesticide controls, yet found little reductions in biodiversity in others.

More recent research on aquatic environments has sparked intense interest in the impact of GM (Bt) crops on aquatic invertebrates *Daphnia magna* (Bøhn, 2008), and Trichoptera species (Rosi-Marshall, 2007). These publications warrant future study, given the potential load of novel target proteins that may end up in agricultural runoff and end up in aquatic environments. Further, Douville et al. (2007) present evidence of the persistence of the transgenic insecticidal protein Cry1Ab in aquatic environments and suggest that that sustain release of this bioactive compound in Bt maize production could result in negative impact on aquatic biodiversity.

Impacts on soil microflora and fauna, including earthworms (Zwahlen, 2002), mycrorhizal fungi (Castaldini et al. 2005) and microarthropods in response to Cry endotoxins have also been reported (Wandeler et al 2002, Griffiths et al 2006, Cortet et al 2007).

The significance of tritrophic effects of accumulation of, particularly of insecticidal Cry toxins (Harwood et al 2005, Obrist et al 2006) however is yet to be firmly established. Subchronic dosages of Cry proteins have been demonstrated to affect both foraging behavior and learning ability in non-target bees (Ramirez-Romero et al 2008), and may have indirect effects on recipient populations on other species. The evolutionary implications in terms of fitness are unclear.

A1.2: Unintended direct effects of insect resistance (Bt) and herbicide tolerance genes on the sustainable use of biological diversity related to crop plants and their progenitors, important for sustainable agricultural production and food security

Another important consideration is the adverse effect that certain GM crops may pose for the sustainable use of important crop agrobiodiversity (Gepts and Papas 2003, Quist

2007). Little research to date has been conducted on the evolutionary implications of gene flow from GM crops to wild relatives or landraces. However increased seed production in wild sunflower with introduced Bt genes by Snow et al (2003). that the researchers further found that hybrids of Bt and non-Bt sunflowers had up to 55% more seeds compared to the wild type when the target pest insect was found in the environment, meaning that there was a clear fitness advantage of the potentially weedy hybrid. This shows the potential of Bt-transgenic varieties or hybrids to outcompete native varieties and bring a reduction in diversity from more genetically homogenous domesticated varieties. Outcrossing between Bt and non-Bt plants is also shown in rice in China by Rong et al (2005), the transfer of herbicide tolerance from herbicide tolerate oil seed rape (canola) to weed *Brassica napus* by Mikkelsen (2006) and the expression of Bt and herbicide tolerant proteins in Mexican maize landraces by Dyer et al (2009). This work presents broad evidence of the occurrence of transgene flow. Further, modeling studies by Haygood and Andow (2003) suggest that under recurrent propagule pressure, transgene establishment within a population can occur even under negative selection. With the evidence of broad transgene flow, further work on the evolutionary implications for the sustainable use of biodiversity is warranted.

A2.1 Combinatorial and/or synergistic indirect effects of LMOs with stacked traits or multiple LMOs

The recent development and commercialization of LMOs with multiple transgenic traits has prompted an interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes to endogenous or introduced traits and functions. The indirect effect of such changes may impact the sustainable development of future LMOs, and comes with high uncertainty of other unintended effects that will need to be monitored in the future.

In the case of simultaneous exposure to different classes of Cry proteins introduced in tandem, despite different modes of insecticidal activity, Tabashnik et al (2009) found evidence of cross reactivity among “pyramided” (stacked events) of Cry1Ac and Cry2B endotoxins in transgenic cotton. The cross reactivity led to higher rates of resistance evolution in pink bollworm, Pectinophora *gossypiella,* in a laboratory setting. Their results suggests that in the case of different Cry protein species, cross reactivity between them may confer increased rates of insect resistance the would alter the efficacy and perhaps biological activity of the LMO.

Then (2009) reviews and discusses the evidence for changes in activity and specificity of Bt proteins dependent on synergisitc interactions with extrinsic features. Such changes may critically influence the bioactivity and hence the potential for unintended effects.

Combinatorial, synergistic effects must be carefully considered in the development and risk assessment of stacked event LMOs with respect to the implications on biodiversity and evolutionary consequences for crop genetic diversity. This will be an important area of investigation for risk research, as multi-trait (stacked) LMOs are poised to replace the current generations of GM crops used in global agriculture. More research in this area is needed.

B Scientific information "taking also into account risks to human health", including direct and indirect effects.

The gaps of knowledge concerning human and animal health impacts of LMOs are quite large (Heinemann and Traavik, 2004). In reality, very few LMOs have been tested on humans (Tayabali et al, 2000). Clinical acute toxicity studies are not the same as chronic exposures likely in the use of GM crops, and may not necessarily uncover undesirable effects. Given the ethical and experimental difficulties in testing of substances on human subjects, other mammal species, such as mice and rats, are often used as surrogates for appraising potential human health impacts of LMOs.

Further, with risk appraisal in mind, one must consider that degree of exposures to GM foods will be different depending on the country. That is, the risk factors for Belgians will be different from say, Zambians, due to large differences consumption patterns of maize.

B1.1 Direct effects of target proteins on animal and human health.

A recent publication by Dona and Arvanitoyannis (2009) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is strong evidence for health concerns on many fronts, exposure duration many have not been long enough to uncover important effects and studies should also include subjects with immunodeficiency or exposed to other stress agents.

Bt Cry toxins

A number of studies have raised questions over the possible toxic or immunogenic effects of Cry proteins on mammals (Ito et al 2004, Vázquez-Padrón et al 2000). Further, cytotoxic effects were found in some cases may be tissue specific, meaning effects may be underestimated if the incorrect tissue type is selected for the assay.

Seralini et al. 2007 reviewed data from a feeding trial of MON863 by the producer, which concluded no toxicity, and found evidence for liver and kidney toxicity in rats fed MON863 Bt maize. While the conclusions of Seralini et al were rejected by the developer of the data, the case illustrates that their poor study design, or inappropriate statistical methods applied to scientific evaluations can lead to important effects to go undetected.

Kilic and Akay (2008) report a significant difference (up to 10%) granular degeneration in the kidneys of rats fed Bt vs. non-Bt maize.

Immunological effects have largely focused on potential allergenicity of LMOs, rather than broader suites of immunogenic response. Inhalation studies, rather than oral toxicity are also largely missing from the scientific literature. One study by Kroghsbo et al (2008) found increase antigen-specific antibody response to Bt toxin and PHA-E lectin in a 28 and 90-day study of Wistar rats.

A study by Schroder et al (2007) found a significance difference in white blood cell count and reduced kidney weight among male rats in a 90 day feeding trial with Bt rice.

A team of Austrian researchers conducted feeding trials with a stacked Bt maize event (MON603 x Mon810) and found significant effects vs. non-Bt maize. Along with reports of kidney toxicity, the authors indicate "concluded, that multi-generation studies, especially based on the [reproductive assessment by continuous breeding (RACB)] design are well suited to reveal differences between feeds. The RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions. The RACB trial with its specific design with the repeated use of the parental generation is a demanding biological factor for the maternal organism" (p. 4 Velimirov et al., 2008).

In a 2008 feeding trial on mice with MON810 Bt maize, Finamore et al (2008) conclude: "induced alterations in intestinal and peripheral immune response of weaning and old mice. Although the significance of these data remains to be clarified to establish whether these alterations reflect significant immune dysfunctions, these results suggest the importance of considering the gut and peripheral immune response to the whole GM crop, as well as the age, in the GMO safety evaluation" (Ibid, p. 11537).

Herbicide resistance genes

The effects of a GM vs. non-GM soy diet on the liver of mice were empirically tested in two scientific studies by Malatesta et al. The first study (Malatesta et al 2002) found nuclear modifications in DNA processing in liver cells that may be implicated in metabolic function. In a 2-year feeding study, (Malatesta et al 2008) the researchers observed marked changes in features of liver function, including senescence (ageing) markers and reduced metabolic rates in mice fed GM soybean vs. non-GM soy controls. The authors conclude:

"[T]he present work demonstrate that GM soybean intake can influence the liver morpho-functional features during the physiological process of ageing and, although the mechanisms responsible for such alterations are still unknown and some data have been discussed on a speculative basis, there are several findings underlining the importance to further investigate the long-term consequences of a GM-diet and the potential synergistic effects with ageing, xenobiotics and/or stress conditions. " (Ibid. p. 975)

B1.2 Potential bioactive or toxic effects of emerging classes of LMOs

Schubert (2008) reviews the published literature documenting potential risks to human health posed by the impending introduction of nutritionally enhanced LMOs, designed to produce bioactive molecules, into the food supply. Specifically, Schubert highlights the evidence for the potential production of aberrant transgenic molecules may produce toxic effects or those with profound effects on human development. He concludes that "Without proper epidemiological studies, most types of harm will not be detected, and no such studies have been conducted. " (Ibid p.604)

B2.1Adjuvant response to LMOs, including cross-reactive and recombinatorial effects.

The issue of combinatorial and/or synergistic effect of GM proteins either with endogenous host proteins or with other inserted GM traits (e.g. "stacked" events) is an area of nascent scientific inquiry. Several studies that point towards extrinsic factors may modulate Cry (Bt) efficacy and specificity. For example Broderick et al (2009) found that midgut bacterial presence was required for Cry1Ab insecticidal activity gypsy moth (*Lymantria dispar*) only suggesting the intestinal microflora may modulate toxicity in certain target Lepidopteran insect species. Further, research by Soberon et al (2007) suggest that structural changes to the engineered Cry1Ab protein in cotton may lack important oligmerization feature essential to toxin efficacy towards *P. gossypiella*.

Combinatorial or synergistic effects of recombinant proteins acting as adjuvants[1] to immunostimulatory effects, or as potential allergens is also an area of vigorous scientific inquiry. The protein Cry1Ac has been shown to be immunogenic in mice (Vazquez-Padron, 2000), and produces an adjuvant effect on both mucosal and systemic specific antibody responses (Moreno-Fierros et al 2003, Rojas-Hernandez et al. 2004). In investigations with Cry1Ab protein, Guimaraes et al. (2009) did not find a similar type of adjuvant effect elicited against peanut proteins as with Cry1Ac, yet instead found evidence of Cry1Ab acting as an adjuvant leading to early phase production of leukotrienes and increased Th2 and Th17-cytokine production in branchoalveolar lavage fluids after airway exposure. The implications of possible effects of Cry1Ab to produce allergen-induced cytokine responses are an area of investigation warranting further inquiry.

[1] That is, adjuvancy, the ability of a compound to enhance or facilitate an immune response, particularly sensitization to another (food) protein.

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Attachment 2

This input was submitted to the Norwegian CP-FP and BCH-FP in Norwegian. The key paragraphs have been translated

→ … the advisory board wishes to emphasise that negative effects on biodiversity is somewhat different from negative environmental effects. There is substantial scientific documentation that indicates negative effects of certain LMOs on the environment, e.g. negative effects of Bt-maize on non-target insects. This is not the same as documenting that the use of such LMOs has a negative effect on the functionality of ecosystems, or the total biodiversity. The advisory board experiences uncertainty, broad interpretations and extensive debates with regards to scientific literature and what it tells us about possible effects on biodiversity. Long term studies are also lacking.

The advisory board will not present and discuss the relevant literature but wish to point out that in the recent years a number of relevant scientific studies have introduced new elements for consideration. One example is the work of Ramirez-Romero *et al*. (2008) indicating that when honeybees are exposed to the Cry1Ab toxin through their natural diet (exposure through pollen from GM-plants) this may lead to reduced capacity for learning and altered pattern of feeding. It is therefore not a direct lethal effect but a more subtle effect that may have ecological consequences and an effect on biodiversity due to a possible reduced survival of the species.

When risk assessing LMOs the advisory board wishes to point out that the context of the evaluation is important and that it is necessary to routinely assess the alternatives to any given LMO. Existing agricultural practices with the use of mono cultures and efficient pesticide regimes have had a clear negative effect on biodiversity both past and present. Less weeds, fewer small mammals and reduced access to seeds in the fields have had large consequences for biodiversity – even before LMOs entered the market. An example is the reduction of bird populations which is well studied and documented in Great Britain. When the use of a certain LMO is to be related to biodiversity it is important to consider the consequences of an already established practice and if the LMO contributes to an existing negative trend, if it has a positive effect or if it introduces new elements of risk.

As opposed to many of the existing LMOs on the market there are a number of “new” LMOs that have a large probability of negative impact on biodiversity if released into the environment. These LMOs must therefore undergo a thorough risk assessment. The advisory board would advise caution with regards to:

- GM-viruses with altered traits and host specificity

- GM-fish with cold tolerance/increased growth rate/high tolerance environmental pollutions
- Stress tolerant GM-plants (drought tolerant/cold tolerant)
- GM-plants with a more efficient nutritional uptake
- Pharma plants

Even though these organisms may have what appears to be very useful traits for purposes of production they may also have selective advantages in nature. This could lead to increased invasiveness and change in ecosystem functionality with the consequence that the number of species drop dramatically or that the balance is altered in other undesirable ways. One scenario is the displacement of locally adapted species through spread of stress tolerant GM forage grasses adapted to marginal habitats/growth areas. Another example is the possible consequences of a GM-fish tolerant to higher concentrations of environmental pollutants leading to higher accumulations of pollutants in the food chain which may in turn have negative health effects.

Both in Norway and the rest of the world the case by case approach is a central principle for LMO risk assessment. The advisory board believes this is an important requirement in order to understand the characteristics of each LMO and reveal the possible effects of the intended use. We would in that respect underline the challenges that risk assessors face when dealing with several of the newer LMOs such as GM-trees (long generation span), GM-viruses (may be difficult to control, risk of mutation) and pharmaplants (risk of entering the food chain).

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Attachment 3

Antibiotic resistance marker genes (ARMG)

Relevant scientific reports on the topic of ARMG:

- **EFSA Scientific Opinion (2009)**: Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". EFSA Journal

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With regards to the 2009 EFSA report we would like to make the following comments:

- There are geographical differences in the distribution of the antibiotic resistance genes *nptII* and *aadA* in naturally occurring bacteria and the distribution patterns are often unknown
- We would draw the attention to the mentioned conclusions from the WHO Expert Group on Critically Important Antimicrobials for Human Health regarding the categorization of the antimicrobials kanamycin, neomycin and spectinomycin as 'Highly Important Antimicrobials' and streptomycin as a 'Critically Important Antimicrobial'
- The EFSA opinion had two minority opinions that should be noted

- **VKM (2005)**. An assessment on potential long-term health effects caused by antibiotic resistance marker genes in genetically modified organisms based on antibiotic usage and resistance patterns in Norway. http://www.vkm.no/dav/23de90b2ff.pdf