SUGGESTIONS ON HOW TO APPLY INTERNATIONAL SAFETY TESTING GUIDELINES FOR GENETICALLY MODIFIED ORGANISMS

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Abbreviations

AHTEG	Ad Hoc Technical Expert Group (Cartagena Protocol on Biosafety)
CAC	Codex Alimentarius Commission
CPB	Cartagena Protocol on Biosafety
EC	Expert Committee (advisory to GEAC)
GE	Genetic engineering
GEAC	Genetic Engineering Approval Committee
GM	Genetically modified
GMO	Genetically modified organism
LMO	Living (genetically) modified organism
PCR	Polymerase chain reaction

This report was prepared free of charge as part of the University of Canterbury's obligation to serve as critic and conscience of society, and the Centre of Integrated Research's founding mission to provide research-based advice on issues of biotechnology of importance to the community, particularly for those unable to normally access state funding for research.

The author thanks Dr. B Kurenbach for critical review.

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Recommendation 2: A scientific risk assessment should be based on scientific information that is available for review and verifiable (through independent testing) by qualified scientists who have reliable career independence from the commercial incentives pervading both public and private research.

Recommendation 3: The regulatory review process should follow that outlined in the Flowchart to the Roadmap for risk assessment for the CPB, with particular emphasis on articulating and addressing uncertainty in the assessment and on an integrated approach that builds confidence in the assessment as it progresses.

Recommendation Four: The regulator should reduce uncertainty in the industry about the standards required in the risk assessment process. The most straightforward approach is to establish the minimum expectation that the developer will conduct the risk assessment by addressing each step of the procedures in relevant international guidance documents. In establishing this standard, the regulator should expect a response to every paragraph of the CAC guidelines (and any other chosen risk assessment procedures) rather than picking and choosing *post hoc* which CAC recommendations to pursue.

Recommendation Five: The regulator should review and address all data relevant to the outcome of the risk assessment, regardless of whether the data was obtained for the purpose of the risk assessment.

Recommendation Six: Recommendations 1-5 should be completed before approving field trials or releases into the environment, or approval for use of the product as food.

Preamble

In mid-2011 the Centre for Integrated Research in Biosafety (INBI) at the University of Canterbury received a request from Ms. Aruna Rodriguez to supply for her an analysis of the food safety evaluation conducted by the Genetic Engineering Approval Committee (GEAC) based on the risk assessment conducted by the developers of Bt brinjal event EE1. This follows from previous reviews done by INBI and provided to Ms. Rodriguez and to the then Minister for the Environment and Forestry, Jairam Ramesh.

This report is provided in partial fulfillment of INBI's public-good service mission. The Centre regularly responds to questions received from representatives of civil society. This report is produced on behalf of INBI and is the author's expert opinion.

Concurrent with the production of this report, the Minister for the Environment and Forestry held a meeting with Ms. Rodriguez. That meeting established Terms of Reference for a Supreme Court Expert Committee. Much of this document will also form a submission to that Committee.

Introduction

Consideration for approval for the release and use of genetically modified brinjal products, herein called 'Bt brinjal', was conducted through a multi-stage process in India under the apex regulator called the Genetic Engineering Approval Committee (GEAC). GEAC engaged two expert review committees (EC I and EC II) that were tasked with reviewing the primary data in biosafety studies conducted and supplied by the product's developers and making a recommendation to GEAC. In August 2007, EC I largely accepted the developers' assertion of safety based on their data of Bt brinjal, but made some additional recommendations to be satisfied before commercial approval could be granted.

Between September 2008 and February 2009, the Bt brinjal developers' biosafety dossier was examined and contested by international scientists. The petitioner Aruna Rodriguez challenged GEAC in the Supreme Court on the basis of these appraisals of the developers' dossier. EC II was convened in February 2009 to answer criticisms, from international and Indian scientists, of the conclusions of safety based on the applicants' dossier, as well as concerns expressed from civil society. GEAC accepted the recommendation of EC II, detailed in their report of October 2009, that Bt brinjal be approved for commercial cultivation. The GEAC steadfastly maintained that it had sufficient information to evaluate the safety of Bt brinjal for both human health and environmental release, and that the information provided to the regulator justified GEAC's high confidence that the product was safe for consumption and environmental release. However, Mr. Jairam Ramesh, the then Honourable Minister of the Ministry of Environment and Forests, following a nation-wide outcry, intervened and instituted a review over the next 4 months.

On 9 February 2010, as a result of the review including public hearings, Minister Ramesh announced a moratorium on the release of Bt brinjal. In coming to this decision, the Minister rejected GEAC's advice.

After a careful consideration, the Minister concluded that:

> "it is my duty to adopt a cautious, precautionary principle-based approach and impose a moratorium on the release of Bt-brinjal, till such time independent scientific studies establish, to the satisfaction of both the public and professionals, the safety of the product

from the point of view of its long-term impact on human health and environment, including the rich genetic wealth existing in brinjal in our country.

A moratorium implies rejection of this particular case of release for the time being; it does not, in any way, mean conditional acceptance. This should be clearly understood."

In the Minister's announcement of the moratorium he specifically indicated that the procedures used by GEAC needed to meet higher standards[1].

Box 1: What is Biotechnology vs. Genetic Engineering?

From the Convention on Biodiversity (Biotechnology) and the Cartagena Protocol (Modern Biotechnology): "The term 'biotechnology' refers to any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for a specific use. Biotechnology, in the form of traditional fermentation techniques, has been used for decades to make bread, cheese or beer. It has also been the basis of traditional animal and plant breeding techniques, such as hybridization and the selection of plants and animals with specific characteristics to create, for example, crops which produce higher yields of grain…

'Modern biotechnology' means the application of:

a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or

b. Fusion of cells beyond the taxonomic family,

that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection" (CBD, 2011).

Genetic engineering (GE) is an example of modern biotechnology because it is based on in vitro nucleic acid techniques. GMOs are products of modern biotechnology. In this report, biotechnology is not synonymous with genetic engineering, but is reserved to recognise and value all knowledge contributions to animal husbandry, crop and forest cultivation, and fisheries management.

This report specifically addresses the first and second Terms of Reference for the Expert Committee constituted by the Supreme Court of India to advise it on the matter of open field trials:

1. To review and recommend the nature of and sequencing of risk assessment (environment and health safety) studies that need to be done for all GM crops before they are released into the environment.
2. To recommend the sequencing of these tests in order to specify the point at which Open Field Trials can be permitted.

I hope to constructively contribute to efforts to achieve a risk assessment framework in India based on international guidelines to establish the safe use of products of modern biotechnology (Box 1) as food, feed and for release into the environment. This framework, I argue, will require India to set

[1] For example, paragraph 16 "It does appear that the current standards by which the GEAC has formulated the decision to approve Bt-brinjal do not match these global regulatory norms to which India is a party."

high but appropriate scientific standards for the data provided in support of the safety of this and other GM plants, and to require the same from other countries that export food or living modified organisms (LMOs) to India.

Only when the regulatory procedures and framework are fully transparent and studiously followed will India create an "enabling environment" (p. 1 CBD, 2003) for the products of modern biotechnology, because the advancement of the use of new technologies will proceed even under a rigorous regulatory assessment provided that the industry has appropriate foreknowledge of the regulators' expectations, and that all developers experience consistency in the regulation (Box 2).

Guidance on the risk assessment and risk mitigation process has been published by numerous international bodies, government agencies and academic scholars. This report will draw on them as appropriate. However, the key recommendations of this report will be consistent with the Codex Alimentarius Commission (CAC), which issues international guidance on food safety, and the Cartagena Protocol on Biosafety (CPB), which issues international guidance on environmental risk assessment.

According to the CAC:

"The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. The expected endpoint of such an assessment will be a conclusion regarding whether the new food is as safe as the conventional counterpart taking into account dietary impact of any changes in nutritional content or value. In essence, therefore, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to determine whether any measures are needed and if so to make well-informed and appropriate decisions" (paragraph 21 CAC, 2003a).

According to the Guidance developed and accepted by the Parties to the CPB:

"There are some overarching issues to consider in the design/planning phase of the risk assessment process to ensure the quality and relevance of the information used. These entail, among others:

- Setting criteria for relevancy in the context of a risk assessment – e.g., data may be considered relevant if they can affect the outcome of the risk assessment.
- Establishment of scientifically robust criteria for the inclusion of scientific information" (AHTEG, 2010).

Box 2. Regulation per se does not inhibit the flow of benefits to the poor.

This Committee may hear from others that the cost of regulation inhibits the flow of benefits of the products of modern biotechnology to the farmer and to the poor in two ways: 1) the regulatory burden slows development and limits the number of technologists willing to work in the area; and 2) the safety standards are set so high as to preclude solutions coming from the public sector, leaving the products of modern biotechnology exclusively in the hands of the large agrochemical multinational corporations (e.g., Focus E of WorldBank, 2007). These arguments have been reviewed elsewhere and found wanting for both evidence-based objective evaluation and validity (IAASTD, 2009).

Notably, the public sector develops these products shackled to licenses and material transfer agreements from private and public actors (Heinemann, 2009). In the case of Bt brinjal, this likely includes for example foundational licenses between the public sector institutions in India and Monsanto Corporation headquartered in the United States. Whether recognised or not in developing countries, legacy material transfer agreements and use of processes or products that are recognised by prevailing intellectual property instruments, can be enforced once a country creates domestic legislation consistent with membership in the World Trade Organisation (Cohen et al., 1998, Heinemann, 2007, Heinemann, 2009).

The World Bank was explicit in its findings on this issue:

"With an increasing share of genetic tools and technologies covered by intellectual property protection and largely controlled by a small group of multinational companies, the transaction cost of obtaining material transfer agreements and licenses can slow public research on and release of transgenics" (p. 178 WorldBank, 2007).

The World Health Organisation drew similar conclusions, saying:

"[T]he proliferation of broad patents is thought to impede the research capabilities of other interested parties…The prevailing patent rules have the potential to limit the accessibility of these technologies to public institutions and ultimately poor farmers… This is because if and when researchers in public institutions do get permission to develop the technologies further, access is granted under licence agreements with restrictions on commercializing innovations." (p. 42 WHO, 2005).

Research-based assessments indicate that "[i]ndustrialized countries hold 97% of patents worldwide, and more than 80% of patents granted in developing countries belong to individuals or corporations based in industrialized countries…even pragmatic analysts from the North are concerned that future innovations will be limited under an emerging industry structure where the top five biotechnology firms control more than 95% of gene transfer patents" (p. 3 Sagar et al., 2000).

While safety regulations may be perceived as barriers to the development and release of safe technologies, even if all regulation were removed the prevailing international intellectual property frameworks uniquely applied to products of modern biotechnology (and not breeding in most countries), would be sufficient to restrict development and to price out poor farmers (Heinemann, 2009). "When technology fees for genetically modified (GM) crops are added on, the risk of purchase can often be considered too high for a poor farmer who is also burdened with excessive fertilizer prices and unpredictable rainfall" (p. 15740 Delmer, 2005). Even in the case of "Golden Rice", extensive negotiations from an estimated 32 different patent holders were required to produce the final "humanitarian license" needed for the use of the product in countries with vitamin A deficiencies, and the license would have ongoing implications for any adopting countries should their export rice become contaminated with Golden Rice (Heinemann, in press).

This advice is seemingly vague but for important reasons. It remains the responsibility of the regulator and decision-maker to make the final determination that the data were relevant and their creation was thoroughly scientific. However, a regulator acting without due regard in setting these criteria may experience a backlash. The top-down assertion of regulatory authority will serve to silence many on many issues, but when this top-down approach fails it does so catastrophically, as India has witnessed with Bt brinjal. Therefore civil society leaders and independent scientists should

be resourced participants, financially or in other necessary ways. This is essential to support ongoing dialogues that result in first setting appropriate criteria, then reviewing their operational success, resulting in an enabling and participatory environment for safety regulation. Importantly, in the interest of a sustainable regulatory environment, the practice of some governments that mix trade and industry development goals within the overall goals and responsibility of the regulator should be avoided (Lotter, 2009, Millstone and van Zwanenberg, 2002). The industry, even in poor countries, can fund its participation in these processes with far greater continuity than can the public, and through continuity of contact and other means can build a relationship with the regulator and its officials at many levels (Millstone and van Zwanenberg, 2002). It is a fallacy to believe that any resourcing of civil society will be in "balance" with the industry or those with commercial vested interests. Therefore, in order to set criteria, the process itself should be free of financial or other conflicts of interest, with those who have a vested interest in products invited only to put forth comment on criteria as they are proposed by groups engaged in dialogue with the regulator (Millstone and van Zwanenberg, 2002).

In the meantime, I have considered the quality of the evidence submitted by the developer of Bt brinjal, the quality and currency of the scientific evidence considered by the GEAC, and additional scientific knowledge that is pertinent and/or used by GMO regulatory authorities around the world to put forth comment on what I believe could be considered as a framework for future proposals to use GM crops as food, feed or for release into the environment in India. Those who take the ultimate decision to approve, approve with conditions, or to reject a product of modern biotechnology will draw upon their superior knowledge of India's culture and environment. As with guidance issued by international expert bodies, this guidance is intended to be inspirational. That is, the guidance is meant to assist the regulator and decision maker in identifying the issues of relevance for India.

Bt brinjal - a case study for the Terms of Reference

Bt brinjal is a shorthand for a proprietary derivative of brinjal that has been genetically engineered to express an insecticide, in this case a protein that is toxic to certain kinds of pest insects. 'Bt' is used by some to indicate that the gene for the insecticidal protein is sourced (and subsequently modified) from bacteria found in the soil. These bacteria are *Bacillus thuringiensis* ('Bt'), but the genes are from a large family of genes called *cry*, for the crystal forming proteins that they specify. There are many different *cry* genes and often several different variants can be sourced from a single clonal line of bacteria (Heinemann, 2009, Pigott et al., 2008, WHO, 1999).

Origins of constituents of EE-1

The specific proprietary claim behind Bt brinjal is for an event called EE-1. An event describes the type of recombinant DNA elements used to form one or more transgenes used, and the particular place or places that recombinant DNA has inserted into the brinjal genome. Event EE-1 was claimed to be composed of three transgenes (Mahyco, 2008, Monsanto, 1997):

1. a *cry*1Ac-like gene derived in part from the DNA sequence called *cry*1Ac, the gene for the insecticidal protein (coupled with a version of the heterologous promoter called 35S from the cauliflower mosaic virus);

2. *npt*II, neomycin phosphotransferase II, a gene that confers antibiotic resistance, sourced from a transposable element isolated from bacteria; and

3. *aad*, aminoglycoside adenylyltransferase, another gene for antibiotic resistance sourced from bacteria.

The event was created using the techniques of modern biotechnology to genetically engineer a vector, or plasmid, originally isolated from the plant pathogen and soil bacterium called *Agrobacterium tumefaciens* (so named for its ability to transfer DNA to plants and induce tumour-like cell proliferation). The plasmid was the 11.4 kilobase-pair (kbp) binary plasmid vector PV-LEBK04, also called pMON10518 (Monsanto, 1997). Taken together, PV-LEBK04 has at least 10 different DNA elements that have been taken from different species, including soybeans, viruses, plasmids isolated from different species of bacteria, and many of which have also been extensively and separately subject to *in vitro* modification after being taken from their natural sources (see Table 2 of Monsanto, 1997).

The commercial trait in Bt brinjal is conferred by the Cry1Ac-like derived protein. This protein is not Cry1Ac isolated from natural plasmids of *B. thuringiensis v. kurstaki*, but a protein made from a series of *in vitro* modifications. The first 40% of the amino acids found in Cry1Ac[2] were replaced with 466 amino acids from Cry1Ab, another insecticidal protein. The developer has claimed that the fusion construct is 99.4% identical in amino acid order to the natural Cry1Ac protein (p. 33 of Mahyco, 2008). However, when I construct the fusion using sequences published in Genbank, I find that the fusion is a maximum of 94% identical to Cry1Ac (GenBank: ABD37053.1) and only 95% identical to Cry1Ab (GenBank: ABV91087.1). Based on these matches, it is also not clear why the developers have historically called their fusion construct after *cry*1Ac rather than after *cry*1Ab, or more precisely, *cry*1Ac-like to accurately identify it as a product of modern biotechnology formed as a chimera of multiple origins. Furthermore, the developer reports that a leucine residue at position 766 has been replaced by a serine residue *in planta*.

What appear to be small differences can be physiologically and immunogenically important. The change from leucine to serine at position 766 is of interest to the biosafety investigator because only the latter can be O-linked glycosylated by the addition of N-acetylgalactosamine through a side chain hydroxyl group (Mitra et al., 2006). "O-linked glycosylation has a profound effect on the antigenic properties of peptides. O-linked glycosylation can generate a neo-epitope (e.g., CII), or can have as an effect the hiding of an epitope (e.g., VF13N). O-linked glycosylation can mimic other epitopes (molecular mimicry of cytokeratins). It can change the properties of an epitope even without really being part of the epitope (CD43 and GPA)" (p. 178 Van den Steen et al., 1998).

The implications of this are that the fundamental affinity-based tools used by developers to list and characterise all forms of the protein produced in the plant (e.g., western blots) may be compromised if they were not trained on the glycosylated forms that the plant may produce. All subsequent studies that are based on isolated proteins (e.g., digestibility, toxicity, and cooking studies) may then be invalid because they only have available to them a subset of the relevant protein isoforms that may be in the plant.

At 99.4% identity, there would be approximately 7 amino acid differences between the chimera fusion and natural Cry1Ac (consistent with original description by Monsanto, 1997)[3]. At 94%

[2] Nomenclature: *gene names* are italicised, protein names Capitalised.

[3] EC II described the construct in Bt brinjal as composed of (the first) 466 amino acids from Cry1Ab and 712 amino acids from Cry1Ac (amino acids 1178 to 467). The regulator said in EC II: "This difference of 0.6% is attributed to the difference in presence of one amino acid at position 766 i.e. serine in place of leucine." However, as noted earlier, currently registered sequences for these proteins do not support that they were this

identity (consistent with current GenBank comparison), there could be up to 70 different amino acids. To conclude that a novel protein is likely to be of no safety concern because of even as few differences as 7 amino acids is not a research-based conclusion. Changes of single amino acids can significantly alter the characteristics of proteins (e.g., Doyle and Amasino, 2009, Hanzawa et al., 2005, Zubieta et al., 2008), a fact that underpins the field of directed evolution (reviewed in Bloom and Arnold, 2009, Tracewell and Arnold, 2009). One of the characteristics that can be changed is immunogenicity. For example, several groups reported significant decreases of IgE binding to a major peanut allergen after mutating single nucleotides (King et al., 2005, Ramos et al., 2009). Even more surprising, in some cases even a synonymous (i.e., differences in the nucleotide sequence of a gene that do not alter the resulting amino acid sequence) coding change can alter the characteristics of a transcript or protein, or levels of expression (Parmley and Hurst, 2007). A single nucleotide polymorphism that results in a synonymous change can change the substrate specificity of the resulting protein, potentially by affecting its folding patterns during translation (Kimchi-Sarfaty et al., 2007). Furthermore, sequence identical proteins with differing tertiary structures can turn benign proteins into toxins (Bucciantini et al., 2002, Ellis and Pinheiro, 2002, Ross and Poirier, 2005) or agents that cause pathogenesis as demonstrated for the Prp proteins causing Creutzfeld-Jacob disease and mad cow disease (Caughey and Baron, 2006).

It is common practice to further alter the coding sequence of recombinant DNA at the nucleotide level to replace synonymous codons or improve mRNA stability for optimal expression in plants (Beuning et al., 2001, de Maagd et al., 1999), and the developer indicates that sequence modifications were made to the *cry*1Ac-like gene (p. 33 of Mahyco, 2008). These altered sequences can result in changes at both the transcriptome and proteome levels because the nucleotide sequence *per se* governs interactions between RNA processing elements and the transcript (Parmley and Hurst, 2007). It is of interest to know how extensively the *cry*1Ac-derived fusion construct was modified at the DNA level and test for these kinds of effects.

Characterising EE-1

The developer claimed that there was a single insert into the brinjal genome and that it corresponded to just the *cry*1Ac-like gene DNA intended for insertion as determined by a Southern blot (p. 34 of Mahyco, 2008). There are several reasons why this evidence was incapable of demonstrating the required proof.

1. The only probe used was described as "Bt" (p. 34), presumably meaning the *cry*1Ac-like DNA sequence. Since the probe is specific to only this part of pMON10518, the blot was completely inappropriate for establishing that there are no other inserts and no backbone DNA from pMON10518.

2. No information was provided about sensitivity of the probe. In order to conclude that there were no other inserts of *cry*1Ac-like DNA, the developer would have to have had a control on the gel that showed detection with their probe at a concentration of 0.5 copies of target (*cry*1Ac-like gene) per diploid Bt genome. The developer could have identified a locus with more than one insert in tandem but miss single copy inserts elsewhere in the genome. *This level of reporting is below what would be expected of a properly peer-reviewed scientific finding.*

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similar. Moreover, a single amino acid difference in a sequence of 1178 amino acids would have been 0.1% rather than 0.6% as the scale of difference between the fusion and nature reported by the developer and accepted by EC II. Neither the original Monsanto dossier nor the EC II numbers support the EC II conclusion that the fusion had a single amino acid difference from what is found in nature.

3. The PCR data did not substitute for the required Southern data because small fragments could not be expected to insert in the correct order or proximity for amplification by this technique.

4. The Southern blot image provided by the developer was below acceptable standard for other reasons as well. A light band of the same size could be seen in control lanes and in the lanes with DNA taken from transgenic plants. This result can arise from sloppy handling and loading of samples. It can also result from contamination of control lines. If the latter occurred, then the applicant would have inadvertently used controls that are also GM plants. International food safety guidelines specifically council against the use of GM plants as controls (CAC, 2003a). Since the probe sensitivity was not reported, the possibility that the control lines carried a single simple insert could not be ruled out with these data. In addition, larger bands were seen to hybridize and these could have inserts to which the probe binds. The only way to resolve these possibilities was to clone and sequence all visible bands. This was not done.

5. Critically and most importantly, the developer used a plasmid that was designed to transfer *in toto*. The Monsanto Corporation, which provided the developer with the plasmid, states this plainly: "The *cry*1Ac and *npt*II genes were introduced into plants of commercial tomato variety UC82B using an *Agrobacterium tumefaciens* binary **single border transformation vector**, PV-LEBK04" (emphasis added to quote from p. 14 of 138 Monsanto, 1997). The developer of Bt brinjal, and the two ECs advising GEAC, repeatedly failed to appreciate the significance of the plasmid map itself which clearly showed only a single T-DNA border sequence, and ignored the reports from independent scientists who alerted EC II to this (see Appendix One of this report). This fact alone invalidates the developer's claims that parts of the plasmid could not transfer (e.g., "The ori322 region is present on the plasmid pMON10518, but was not transferred and hence was not present in the genome of Bt brinjal" p. 34 Mahyco, 2008).

In conclusion, there were significant anomalies in the reporting of the constructs and in the characterisation of event EE-1. In addition to the anomalies, it was apparent that critical and fundamental characterisation of the event was not completed, usually because of assumption-based reasoning (e.g., the plasmid does not transfer because the developer thought that it should not) that was often both faulty (e.g., the entire plasmid was designed to transfer) and also not confirmed by readily available science. When such fundamental misunderstandings of the basic tools of the procedure were demonstrated by the developer, seemingly went unchallenged by the regulator, it was very difficult to accept assurances that the other procedures in the evaluation of Bt brinjal could be trusted. The downstream impacts of poor initial characterisation include:

- distrust of the detection method (which ECII claimed without evidence was specific to Bt brinjal at 0.01% (ECII, 2009)), because failure to properly confirm the number and structure of inserts undermines the design of the tools used to confirm transfer of the recombinant DNA through crosses; and

- invalidation of conclusions surrounding unintended changes to the transcriptome or proteome based either on the expectation that there was a single insert of expected structure or the failure to analyse inserts for open reading frames because those inserts went undetected using only a single probe to *cry*1Ac.

‘To review and recommend the nature of and sequencing of risk assessment (environment and health safety) studies that need to be done for all GM crops before they are released into the environment’

Comparative risk assessment, which is the most common form for products of modern biotechnology that are both intended and expected to be nearly equivalent to their natural (conventional) counterparts, is under prevailing international agreements conducted on a case-by-case basis. What this means is that the GM product is feature by feature compared to a scientifically valid “comparator” that has the properties of being:

- not a product of modern biotechnology (see footnote 1 to CAC, 2003b);
- the near iso-genic parental variety (AHTEG, 2010, CAC, 2003a);

and is conducted for each event regardless of the behaviour of similar events or products of modern biotechnology.

It would be far beyond this report to indicate in detail every experiment that might be required for a decision-maker on a case-by-case basis to establish the acceptability of all possible GM crops intended for release into the environment or as food and feed. Therefore, again using the experience of Bt brinjal as a reference, this report will attempt to provide guidance to the Committee about when and why to invoke the recommendations of Codex Alimentarius and other risk assessment procedures. Because of my personal expertise, the emphasis from me will be on molecular characterisation although I will contribute to considerations of what should be applied at other levels of testing, such as toxicity, immunomodulation, and environmental testing. Whereas the particular technical details of each risk assessment may vary from case to case for good and obvious reasons, there are some aspects of the assessment that are generic, have historically been undervalued. No amount of technical sophistication or asserting of authority later will compensate for a failure to properly recognise and respect these overarching issues:

1. Risk assessment itself requires agreement on the nature and scope of the risks to be considered and thus risk assessment authorities must be inclusive, engaging all stakeholders in the formulation of the nature and scope of the assessment.
2. For the component of the risk assessment that is based on technical (scientific) information, that information needs to be trusted. For trust, the following should be considered a minimum:
    - “Data should be of an acceptable scientific quality. Data quality should be consistent with the accepted practices of scientific evidence-gathering and reporting and may include independent review of the methods and designs of studies. Data may be derived from a variety of sources, e.g., new experimental data as well as data from relevant peer reviewed scientific literature” (AHTEG, 2010).
    - The science should be sound. “Sound science is based on transparency, verifiability, and reproducibility (e.g., reporting of methods and data in sufficient detail, so that the resulting data and information could be confirmed independently), and on the accessibility of data (e.g., the availability of relevant, required data or information or, if requested and as appropriate, of sample material), taking into account the provisions of Article 21 of the Protocol on the confidentiality of information. The

provisions of sound science serve to ensure and verify that the risk assessment is carried out in a scientifically sound and transparent manner" (AHTEG, 2010).

3. Uncertainty exists at all levels of a risk assessment, from conceptualising the nature and scope of the risk to the collection of data, and this uncertainty needs to be acknowledged and addressed through ongoing engagement with stakeholders, sound scientific risk mitigation and post-release monitoring even after a decision might be taken to use or release the product of modern biotechnology (AHTEG, 2010, Pavone et al., 2011).

If these overarching issues cannot be satisfied for whatever reason, including the capacity of the country or the demands of the developer for proprietary reasons, it remains the prerogative of the decision-maker to issue a decision against approval for the developer's intended use.

Recommendation 1: The regulatory review process should *begin* with the participation of all stakeholders, from industry (not just the applicant's industry), civil society and government and seek a consensus endorsement in the scope and nature of the risk assessment. The stakeholder engagement should not begin with an evaluation of the outcome of a risk assessment.

Recommendation 2: A scientific risk assessment should be based on scientific information that is available for review and verifiable (through independent testing) by qualified scientists who have reliable career independence from the commercial incentives pervading both public and private research.

Recommendation 3: The regulatory review process should follow that outlined in the Flowchart to the Roadmap for risk assessment for the CPB, with particular emphasis on articulating and addressing uncertainty in the assessment and on an integrated approach that builds confidence in the assessment as it progresses.

Use of risk assessment resources

Conflicting opinions on modern biotechnology products is neither unexpected nor uncommon. Therefore, it is essential for a precautionary regulator to be independent of conflicts, or perceived conflicts, of interest (Millstone and van Zwanenberg, 2002). In recommending Bt brinjal for open cultivation and use as food, GEAC appeared to have lost the confidence of the States of India and its Minister, along with attracting the criticism of many citizens, academics and some from the industry as well. Every regulator must make decisions with which some will disagree. However, in this case the actions of GEAC gave the appearance that the decision was one which the regulator could have come to through a failure of process rather than a difficult choice. Following recommendations 1-3, above, should help to avoid this outcome in the future.

In addition, GEAC can call upon international food safety and environmental safety guidelines (e.g., the CAC, World Health Organisation/UN Food and Agriculture Organisation, CPB), the scientific and other relevant literature, the Roster of Experts (maintained by the Secretariat of the Convention for Biodiversity), a representative diversity of opinion among Indian academics and Non-Governmental Organisations and, finally, farmers themselves. International food safety guidelines are well established, even if their application from country to country is inconsistent. In the case of environmental testing, the CPB also provides broad guidance. There exists a considerable literature for testing environmental impacts of GMOs (for a recent review, see BAT) and there are mechanisms for seeking additional help (e.g., through the Roster of Experts).

Annex III of the CPB advises that:

> "Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations."

The CPB does not limit the regulator, but empowers the regulator to institute true transparency and to require sound science. While the CPB does not specify that India should require a particular experiment to be done, having considered either the need for some kind of experiment or the quality of the data provided, the regulator would be fully within its rights to withhold a favourable judgement until that experiment was done, done to a proper standard, and properly presented to the regulator for review. For example, there were many relevant risks pointed out by both the independent scientific community and by members of the GEAC[4]. Under the Protocol, these could have been pursued by asking for more relevant data:

> "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."

Critically, a regulator should be supported either by its own internal capacity to perform safety testing or by a specialist community of fundamental safety researchers with clear career pathways that allow them to work without the need for industry or political associations for reasons that have been made clear by the research community (Graff et al., 2003, Lotter, 2009, Millstone and van Zwanenberg, 2002, Shorett et al., 2003). This is presently not the case, and not just in India. Professional entanglement with industry, including but not limited to direct access to industry funding, has been identified as a powerful influence on the conclusions drawn on the safety of GMOs.

> "[A]rticles where a [conflict of interest] was identified show a tendency to produce outcomes favorable to the associated commercial interests. These results support the overall view that all affiliations should be clearly acknowledged in scientific publications on the risk analysis of GM food or feed products, as the existence of such conflicts of interest is somehow interfering with study outcomes" (p. 201 Diels et al., 2011).

> "Career considerations, long standing personal scientific viewpoints, or value-based opinions over the role of science in society and faith in technology as a useful tool for solving global problems, may influence author perceptions and study outcomes" (p. 202 Diels et al., 2011).

Moreover, food safety regulators around the world depend on data submitted by the developer, with the inherent conflict of interest this implies (Millstone and van Zwanenberg, 2002). It is a system that needs to change and the problems with this system have become most visible over Bt brinjal in India.

Proper 'risk assessment' for both food and environmental safety includes competent and rigorous hazard identification, followed by safety testing, risk assessment and then, if appropriate, risk management (AHTEG, 2010, BAT). The key to proper hazard identification is confidence in the approach for effectively testing any expected potentially adverse effects of the plant and, importantly,

[4] For example, Dr. MS Swaminathan called for tests to detect potential chronic effects of eating Bt brinjal, of the type used to establish the causes of cancer due to smoking cigarettes. Letter from MS Swaminathan to the Minister, dated 4 February 2010).

discovering any unintended or unanticipated adverse effects that might arise from the modification or the process of creating the GM plant. As stated in the CAC Principles (CAC, 2003b):

> "A safety assessment is characterized by an assessment of a whole food or a component thereof relative to the appropriate conventional counterpart:
>
> A) taking into account both intended and unintended effects;
>
> B) identifying new or altered hazards;
>
> C) identifying changes, relevant to human health, in key nutrients" (paragraph 11).
>
> "Safety assessment should include data and information to reduce the possibility that a food derived from a recombinant-DNA plant would have an unexpected, adverse effect on human health" (paragraph 14 of CAC, 2003a).

Applying CAC guidelines to Bt brinjal

The guidance provided by the World Health Organisation (WHO) and the UN Food and Agriculture Organisation (UN FAO), and the CAC varies from specific recommendations for experimental procedures (e.g., number of contiguous amino acids matching between a novel protein and a known allergen, specific controls in a protein digestibility assay), to higher level indications of what kinds of tests might be useful. The guidance then leaves it to the regulator to indicate what of this information is required to issue a decision of acceptably low uncertainty about safety. How the regulator makes use of this guidance has a significant impact on the trust stakeholders place in the regulator's process. I will illustrate, with the CAC guidelines, how a process intended to instil trust might be conducted (Table 1).

It is important to note that the international guidelines produced by the CAC and by the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management of the CPB are already 'compromise' documents formulated using contributors from government agencies (even from governments that may not be parties to the relevant international agreements), United Nations agencies, the industry being regulated and observers. All contributors regardless of whom they represent in text negotiations may have potential conflicts of interest[5] (Millstone and van Zwanenberg, 2002). The CAC also has historically been placed in a role of trade promotion (Millstone and van Zwanenberg, 2002). The guidance produced by these international bodies represent the minimum common denominator of safety advice that will protect a state from trade-related challenges (Millstone and van Zwanenberg, 2002).

I raise this issue for two reasons. Firstly, I wish to highlight that the guidance is not extreme in its formulation. Indeed these documents had strong representation from the industry and governments that promote the modern biotechnology industries. Secondly, there is the danger of further diluting the effectiveness of the guidance by "picking and choosing" the standards applied at the national level, especially when at this level the regulator or its expert committees may also have the kinds of conflicts of interest discussed by Millstone and van Zwanenberg (2002).

[5] For membership in the Ad Hoc Technical Expert Group, see UNEP/CBD/BS/COP-MOP/5/12. "At Codex meetings decisions are taken by national delegations, but those delegations have sometimes been headed by trade promotion officials rather than food safety officials and national delegations often include large numbers of representatives of the commercial sector, with direct interests in the decisions they take" (p. 595 of Millstone and van Zwanenberg 2002).

In the table that follows, the use or otherwise of the various CAC guidelines is illustrated for Bt brinjal.

Table 1: Selected standards allowed by CAC guidelines.

CAC guideline	Evaluation using the Bt brinjal risk assessment as a case study
paragraph 18 (and 22-26 and 34, not reproduced) The safety assessment of a food derived from a recombinant-DNA plant follows a stepwise process of addressing relevant factors that include: A) Description of the recombinant-DNA plant; B) Description of the host plant and its use as food; C) Description of the donor organism(s); D) Description of the genetic modification(s); E) Characterization of the genetic modification(s); F) Safety assessment: a) expressed substances (non-nucleic acid substances); b) compositional analyses of key components; c) evaluation of metabolites ; d) food processing; e) nutritional modification; and G) Other considerations.	While some information on this was provided in the various expert evaluations of the Bt brinjal data, as discussed above, in important categories the information was technically flawed, derived from unverified assumption-based reasoning, or missing. The regulator would have been justified to apply higher standards of reporting.

paragraph 20 Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.	The reports should have described all experimental procedures as necessary for the experiment to be conducted independently. This was not done.
Points to consider Failure to provide this information is not acting in a transparent way, prevents independent verification and reproducibility.	
paragraph 21 The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use.	The regulator should have a process in place that will engage all relevant stakeholders to establish the nature and scope of testing that will be required. Then the regulator should guide the process to ensure ongoing engagement by the group and enlarge the group as appropriate during the course of the assessment. This early and continuous engagement was lacking in the Bt brinjal process.
Points to consider Failure to agree on nature and scope of the assessment undermines confidence in the outcome, as demonstrated by Bt brinjal.	
paragraph 25 (and paragraphs 35, 38, 41-43 and 49 not reproduced) The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (e.g., which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).	Demographically relevant information should be developed. This includes variance in exposure according to cultural diversity (e.g., medicinal use) and according to age and sex. The demographic analysis should inform testing for maximum exposure and for such analyses as digestibility, anti-nutrients and immunomodulation. The Bt brinjal evaluation was based on contested generalisations about how brinjal was consumed in India, drew conclusions based on comparisons to other products and not the Bt brinjal itself, and had studies that did not

confirm the Cry protein content in the food.
Points to consider 1. Average exposures are not informative when it comes to food. Even within apparently homogenous societies, maximum exposures can be significantly underestimated. Importantly, the physiology of humans and animals (wildlife, 'nontarget' insects, etc.) can vary with age, the potential adverse effect such as allergenicity or toxicity may be age-specific. 2. Testing that can be affected by variations in physiological state of exposed groups must be customised. Recall as well that this is consistent with the advice from the Roadmap prepared for Annex III of the CPB ("data may be considered relevant if they can affect the outcome of the risk assessment"). For example, novel proteins that are or may be expressed in the GMO are commonly subjected to an in vitro solution intended to mimic the human digestive system. The stability of the protein is taken as an indicator of the potential for the proteins to persist and thus as an indicator of the potential for the immune system to be exposed to the proteins or their degradation products (Fu, 2002)[6]. International guidelines for the conduct of the digestibility assay recommend that it be conducted using proteins in their edible forms in a solution of representative acidity levels and concentrations of digestive enzymes and that the assay include controls consisting of known protein allergens to gauge the relative stability of the novel proteins (FAO/WHO, 2001). Physiological variation in digestive proteins and stomach pH are common, with babies as rule having higher stomach pH than adults. Adults have higher stomach pH as they progress through a meal. Depending on the concentration of digestive proteins and pH, different proteins reveal different potential allergens (Morena, 2007). Thus, a properly conducted digestive study will be based on a previous analysis identifying maximum exposures by age, sex and ethnicity and the conditions of the digestive study will be constructed to provide relevant data for these groups. 3. Testing should be relevant to kind of exposure. Allergen exposure occurs through ingestion, contact and inhalation. Digestibility and bioinformatic studies are not sufficient to exclude allergenicity from tall exposure routes (Spok et al., 2005). In the case of Cry proteins, inhalation is a potent route of sensitisation (Kroghsbo et al., 2008). In the case of Bt brinjal, GEAC accepted from the developer evidence from only single condition digestibility studies (pepsin at pH 1.2 and trypsin at pH

[6] Note that the digestibility assay cannot prove that a protein will not be an allergen because many confirmed allergens are highly unstable in the *in vitro* digestibility assay. However, stability through a proper assay is evidence that the protein may cause an adverse effect and further testing should be conducted to counter this suspicion. It "is widely accepted that a protein that is resistant to digestion would have an increased probability of stimulating allergic reactions; in vitro digestion assays provide an estimate of the relative integrity of a protein and thus the probability of eliciting the allergic reactions" (p. 107 of Fu 2002). Likewise, failure to find regions of amino acid sequence similarity between novel proteins and known allergens is not proof that the novel protein, when used in food, will not be an allergen. However, matches indicate that further testing is required to ensure a protein is not an allergen.

7.5), did not report digestive enzyme to protein ratios, and used a surrogate source for the protein instead of the protein isolated from Bt brinjal event EE-1 in its edible form (ECII, 2009, Seralini, 2009). Furthermore, a digestion-resistant fragment was reported as very stable. This observation alone could justify additional testing. The bioinformatic analysis was conducted 14 years prior, reported to be against a 1994 database of known allergens conducted by Monsanto and contained in an unpublished internal report (p. 106 of Mahyco, 2008). Essentially the same limited analysis was used to assess the allergenicity potential of NptII (Mahyco, 2008). No tests of inhalation exposure were conducted on either protein.	
paragraph 27 (and 28-29, not reproduced) Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted in the plant.	While some information on this was provided by the Bt brinjal developers, as discussed above, the information was technically flawed and/or derived from unverified assumption-based reasoning.
paragraphs 30-32 In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant-DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out. (31) Information should be provided on the DNA insertions into the plant genome; this should include: A) the characterization and description of the inserted genetic materials; B) the number of insertion sites; C) the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate, other information such as analysis of transcripts or expression products to	While some information on this was provided, as discussed above, the information was technically flawed and/or derived from unverified assumption-based reasoning. Information that could be requested is based on techniques that produce descriptions of novel changes to the transcriptome and proteome (for novel RNA or protein molecules, or novel concentrations of normal RNA or protein molecules) for purposes of hazard identification (Heinemann et al., 2011). This is especially relevant as it has now been confirmed that small RNA molecules from food can transfer to the human circulatory system, and are capable of altering gene expression patterns in mammalian organs (Zhang et al., 2011). If changes unique to the GM plant are found, these unexpected or unintended changes can be further assessed if necessary.

identify any new substances that may be present in the food; and

D) identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

(32) Information should be provided on any expressed substances in the recombinant-DNA plant; this should include:

A) the gene product(s) (e.g. a protein or an untranslated RNA);

B) the gene product(s)' function;

C) the phenotypic description of the new trait(s);

D) the level and site of expression in the plant of the expressed gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions; and

E) where possible, the amount of the target gene product(s) if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.

Points to consider

This type of information is often considered the most basic component of the molecular characterisation. It is critical to perform to high standard because failing to do so can compromise the effectiveness of later stages of the assessment in profound ways (Heinemann et al., 2011). In addition, the information is necessary to address the source of potential adverse effects that can arise from unintended changes, as discussed in paragraphs 15-17.

(15) "Unintended effects can result from the random insertion of DNA sequences into the plant genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of enzymes at high levels may give rise to secondary biochemical effects or changes in the regulation of metabolic pathways and/or altered levels of metabolites.

(16) “Unintended effects due to genetic modification may be subdivided into two groups: those that are ‘predictable’ and those that are ‘unexpected’. Many unintended effects are largely predictable based on knowledge of the inserted trait and its metabolic connections or of the site of insertion. Due to the expanding information on plant genome and the increased specificity in terms of genetic materials introduced through recombinant-DNA techniques compared with other forms of plant breeding, it may become easier to predict unintended effects of a particular modification. **Molecular biological and biochemical techniques can also be used to analyse potential changes at the level of gene transcription and message translation that could lead to unintended effects** (emphasis added).

(17) “The safety assessment of foods derived from recombinant-DNA plants involves methods to identify and detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. **A variety of data and information are necessary to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to human health**…” (emphasis added).

As indicated earlier in this report, there were a number of sources of potential unintended inserts and gene products in Bt brinjal. These included:

- undetected inserts because of poor experimental procedure, and failure to verify often faulty assumptions about the gene transfer process;
- undetected alternative mRNAs or non-coding RNAs that could have arisen from: undetected inserts or undetected new open reading frames; alternative splicing of both intended and unintended novel RNAs because of recoding of the various genes in EE-1; unintended entry of intended or unintended novel RNAs into double-stranded RNA processing pathways that could lead to unintended and unpredictable activation or silencing of other genes, including those that might lead to higher levels of anti-nutrients, toxins or allergens;
- undetected and unintended proteins or isoforms of proteins that arise from: post-translational modifications of intended proteins (e.g., forms of the Cry1Ac-derived fusion protein glycosylated at the novel serine but present at low concentration); novel small or large proteins from unintended mRNAs and these same as unintended and unpredictable post-translationally modified isoforms; collectively these could contribute to new or higher levels of anti-nutrients, toxins or allergens.

While anti-nutrients, toxins and allergens are of course important to consider when Bt brinjal is used as food, taken together these same hazards may impact on an environmental risk assessment where they might manifest as unintended adverse effects to wildlife, important insects or to exposed microbial communities.

paragraph 33	There were significant methodological flaws in the information provided with relevance to paragraph 33. Using targeted techniques for the isolation

In addition, information should be provided: A) to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration; B) to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function; C) to demonstrate whether the intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly; D) to demonstrate whether the newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene; E) to indicate whether there is any evidence to suggest that one or several genes in the host plant has been affected by the transformation process; and F) to confirm the identity and expression pattern of any new fusion proteins.	of in-planta produced novel protein, the regulator could require the developer to measure concentration in a tissue and time of development study across multiple test sites and years. Molecular mass of all isoforms using mass spectrometry could be measured. Finally, the regulator could ask for toxicity, immunomodulation, allergenicity or adverse effects testing on wildlife, insects and microbes.
paragraph 37 (and 38, not reproduced) [C]onventional toxicology studies may not be considered necessary where the substance or a closely related substance has, taking into account its function and exposure, been consumed safely in food. In other cases, the	See “Points to consider”, below.

use of appropriate conventional toxicology or other studies on the new substance may be necessary.

Points to consider

The regulator may be tempted in cases of "Bt" or herbicide tolerance traits to underestimate the importance of toxicology testing because these are the most common traits in existing GM plants. As extensively documented in the literature, categorising genes such as the *cry*1Ac-like derivative as having been for some time safely consumed in food is assumption-based reasoning with faulty premises.

Firstly, the use of Cry proteins in *Bacillus*-based insecticides does not routinely leave high residue levels on plants (WHO, 1999) unlike its expression directly in the plant. There is no historical evidence of mass exposure to these proteins through either food or inhalation in the general population, and certainly no evidence of exposures at concentrations that are common in GM plants (Heinemann, 2009).

Secondly, existing commercial GM plants engineered with Cry proteins are intended for use as animal feed. Thus, the exposure to the *in vitro* modified Cry proteins is at present very low in the human diet, even in the United States. Even according to industry figures (James, 2011), significant acreage of GM plants has only been achieved in the last few of the nearly 15 years since first commercialisation. Exposure is ramping up, but any possible long-term effects likely would not yet be detected. This is in contrast to the intended use of Bt brinjal as food and the real possibility of high human exposures.

Thirdly, there is no post-market health surveillance, so there is no reliable way adverse effects could be detected and subsequently inform regulators who in turn are not accumulating objective experience with the use of Cry proteins in human foods.

Fourthly, there is no "control" population in which to compare to assist in detection of potential adverse effects of Cry proteins in human food. The types of plants that have been genetically engineered are suited to use in processed foods and these are rapidly transported around the world. Thus most humans on the planet are being exposed to very low but chronic doses of GM plants.

Fifthly, there is accumulating evidence of potential adverse effects of GM plants producing Cry proteins, and there is documented peer-reviewed evidence that feeding trials that regulators have been relying upon were inappropriately designed to reduce uncertainty about the safety of using these crops as food (Carman, 2010, Gallagher, 2010, Heinemann, 2009, Mesnage et al., 2012, Séralini et al., 2007, Séralini et al., 2009, Seralini et al., 2011).

Finally, there has been no published testing using cooked and processed ingredients made from Bt plants and prepared as humans and not farm animals would eat them. While the developer heated and cooked Bt brinjal, it only tested for presence of the Cry protein (using undisclosed reagents with unverified sensitivity and effectiveness), which is not the same as testing for adverse effects.

paragraph 39 (and paragraphs 40 and 46, not reproduced) Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the plant of the substance and dietary exposure. The type of studies to be performed may include studies on metabolism, toxicokinetics, sub-chronic toxicity, chronic toxicity/carcino-genicity, reproduction and development toxicity according to the traditional toxicological approach.	The diversity of transcripts arising from the intended insertion and any potential unintended insertions will be unique to each GM plant and will have no history of safe consumption by humans. These small molecules do transfer to humans through food (Zhang et al., 2011). Likewise, any unexpected secondary activities of the proteins expressed may create new or alter existing metabolites. Information that could be requested is based on techniques that produce descriptions of novel changes to the transcriptome, proteome (for novel RNA or protein molecules, or novel concentrations of normal RNA or protein molecules) and metabolome for purposes of hazard identification (Heinemann et al., 2011).
paragraph 44 Analyses of concentrations of key components of the recombinant-DNA plant and, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant-DNA plant grown under its expected agronomic conditions may need to be considered (e.g. application of an herbicide). The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be the near isogenic parental line. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.	The absence of a compositional analysis was initially overlooked but then required by EC II after the regulator was forced to re-evaluate its recommendation to the Minister.
paragraph 47 (and paragraph 53, not reproduced) The potential effects of food processing, including home preparation, on foods derived from recombinant-DNA plants should also be considered.	Feeding, compositional, toxicological and allergenicity studies using both whole food and food processed or prepared as humans would eat it should be provided by the developer. A limited analysis was provided for Bt

For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.	brinjal. If adverse effects are noted with the food in this form, then require further testing or reject on safety grounds.
Points to consider As stated above, the developer only monitored for stability of the intended protein in cooked products, not for potential adverse effects of Bt brinjal as a result of any unintended and unpredicted effects. Moreover, the techniques and reagents used were not reported in sufficient detail to evaluate their effectiveness. Feeding studies should be conducted and designed for the purposes of detecting unintended acute or chronic toxicity, immunomodulatory activities, or multigenerational effects (Carman, 2010, Gallagher, 2010, Seralini, 2009, Séralini et al., 2009, Seralini et al., 2011, Velimirov et al., 2008). Wherever possible, the source of the food should be whole food derived from the GM plant (or product if not a plant) which was produced under expected commercial conditions (EFSA, 2008). That is, if the food is derived from a GM plant intended to be treated with a particular chemical herbicide, the feeding study should include material exposed to the herbicide.	
Annex paragraph 17 As scientific knowledge and technology evolves, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. These methods should be scientifically sound and may include targeted serum screening (i.e., the assessment of binding to IgE in sera of individuals with clinically validated allergic responses to broadly-related categories of foods); the development of international serum banks; use of animal models; and examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens	The developer reported comparisons between Cry1Ac only and those were conducted against databases available over 15 years ago. Should follow-up with updated databases and inhalation studies.

Points to consider

According to the developer, the Bt brinjal was cooked on an open flame, deep fried or steamed for various amounts of time. Then an attempt was made to extract only the Cry1Ac-derived protein (and not other proteins of the event (i.e., Aad or NptII)) from cooked material. This assay fails to meet the spirit of the CAC recommendation in several ways:

- no methods, reagents or limits of detection are reported for either the extraction or ELISA. The failure to report on reagents is particularly problematic because these reagents, if affinity based, might be compromised for the detection of heated Cry1Ac.
- the cooked material was not used in feeding and allergenicity studies. This may be important to ensure that neither the intended nor unintended proteins, nor unintended metabolites arising from the modification or the process of creating Bt brinjal, were capable of reacting under normal cooking conditions to create anti-nutrients, toxins or immunomodulating compounds. Cooking and processing are associated with altering, even enhancing, the immunomodulating potential of some allergens. For example:

  "In contrast to these so-called pollen-related allergens, roasting has been reported to increase the allergenicity of raw peanuts. For example, protein extracts of thermally treated peanuts have been shown to bind IgE antibodies from patients' sera at up to 90-fold higher levels than extracts obtained from the corresponding nontreated peanuts. In addition, inhibitory ELISA experiments revealed a significant increase in the IgE binding activity of the purified major allergens Ara h 1 and Ara h 2 after thermal treatment in the presence of carbohydrates" (p. 2290 Gruber et al., 2005).

  In this example, even the minor allergen Ara H 1/2 (peanut agglutinin) was converted into an IgE-binding product after incubation with sugar at elevated temperatures (Gruber et al., 2005).

The developers did conduct a risk assessment that included some elements commonly found in other risk assessments of GM crops (a notable exception being a compositional analysis that was later required by EC II). Most of this content was extremely similar, sometimes word for word identical, to the text composed earlier by the Monsanto Corporation in a older petition to the United States Department of Agriculture requesting deregulation of a tomato line genetically engineered to express the same Cry1Ac-like protein (Monsanto, 1997). The Monsanto application was written in 1997 and thus draws upon literature from the early 1990s, and usually much older. This perhaps helps to explain why many key references are so far out of date in the developer's dossier on Bt brinjal, supplied to the GEAC in 2008. However, it does not excuse the developer or the regulator from having not updated their own knowledge as the scientific understanding of the tools being used.

In general terms, the developer's risk assessment was technically consistent with paragraphs 21, 23 and 26 of the CAC guidelines (CAC, 2003a), as well as parts of other paragraphs. But a risk assessment is not a checklist of compliance, especially when compliance is enriched for largely descriptive elements of the assessment and "cherry picked" rather than representative of the full advice provided in the guidance. Instead, the risk assessment is a dynamic process that should upon completion build a scientific case that potential hazards, if present, would have been found. It should build confidence that safety of the product was by design. For example, paragraph 12 of the Principles says:

> "A pre-market safety assessment should be undertaken following a structured and integrated approach and be performed on a case-by-case basis. The data and information, based on sound science, obtained using appropriate methods and analysed using appropriate statistical techniques, should be of a quality and, as appropriate, of quantity that would withstand scientific peer review" (CAC, 2003b).

And paragraph 20 of the Guidelines says:

> "The goal of each safety assessment is to provide assurance, in the light of the **best available scientific knowledge**, that the food does not cause harm when prepared, used and/or eaten according to its intended use (emphasis added to CAC, 2003a).

Drawing upon text written over 10 years prior and which cited work for conclusions long since expired (see above) does not suggest that the risk assessment was conducted using the best available scientific knowledge. The quality of the data as reviewed previously by the independent scientific community - particularly the lack of methodological detail on everything from the genotype(s) of the comparators used, to the molecular reagents and their origins (Appendix One and Appendix Two of this report), to the design and then statistical analysis of the feeding studies (Carman, 2010, Gallagher, 2010, Seralini, 2009) and finally through to the environmental risk assessment (Andow, 2010, Gurian-Sherman, 2010) - indicates that these studies could not pass review by scientific peers disinterested in the commercial case for Bt brinjal.

Recommendation Four: The regulator should reduce uncertainty in the industry about the standards required in the risk assessment process. The most straightforward approach is to establish the minimum expectation that the developer will conduct the risk assessment by addressing each step of the procedures in relevant international guidance documents. In establishing this standard, the regulator should expect a response to every paragraph of the CAC guidelines (and any other chosen risk assessment procedures) rather than picking and choosing *post hoc* which CAC recommendations to pursue.

Recommendation Five: The regulator should review and address all data relevant to the outcome of the risk assessment, regardless of whether the data was obtained for the purpose of the risk assessment.

In determining whether the best scientific information available has been used for the risk assessment, the regulator should note this advice from the CPB Roadmap: "data may be considered relevant if they can affect the outcome of the risk assessment" (AHTEG, 2010). Provided that the data meet the criteria of being transparent, verifiable and reproducible by independent scientists, then the key feature for emphasis is only relevance of the data to the risk assessment and not the various side-issues such as "industry validation" (Heinemann et al., 2011).

'To recommend the sequencing of these tests in order to specify the point at which open field trials can be permitted'

In general, Recommendations 1-5 normally would be completed before open field trials are recommended. The outcome of the tests and their evaluation must be that there is no indication of adverse effects, or of adverse effects that cannot be made acceptable through risk mitigation strategies.

Recommendation Six: Recommendations 1-5 should be completed before approving field trials or releases into the environment, or approval for use of the product as food.

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