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Mr. Jairam Ramesh
Minister, Environment and Forests
Government of India

Re: Bt-brinjal Part Two (of two)

Dear Minister Ramesh

It is my pleasure to respond to your request of 7 November 2009 for my views on the Mahyco et al. application to approve open cultivation of Bt-brinjal for use as food. As you know, I was one of a number of independent scientists who read and critiqued the scientific information provided by the developers in their case to demonstrate that the genetically engineered Bt-brinjal posed no safety concerns for human health or the environment. I came to the conclusion that the molecular data, and some related analyses, were too poorly conducted to make a definitive claim that this product is safe.

Since that time, the official Indian advisory body, the Genetic Engineering Approval Committee (GEAC), has ruled that the science provided by the company does satisfy them that Bt-brinjal is safe. Later I learned that new information had been released in November of this year. This put additional stress on my ability to meet your request of information by 31 December. I have done everything I could to meet the deadline regardless, but I ask your indulgence to submit more information in the future should I have overlooked something due to the short time available to review the new information.

As promised on 24 November, this is the second part of my response. In this letter, I will review selected criticisms formed in August 2008 when I reviewed the scientific information available at the time, and indicate how these have been addressed by the developers or GEAC.

Summary of analysis of dossier from Mahyco et al. in support of their claims of safety of fruit and shoot borer tolerant brinjal

I previously provided a review of selected topics within Mahyco's dossier. This was referred to in the "Report of the Expert Committee (EC-II) on BT Brinjal Event EE-1" (EC-II) of 8 October 2009 as item 17 "comments by Prof. Jack A. Heinemann".

GEAC considered my comments, as I understand, through the report of the expert committee. However, several significant issues remain either not addressed, misunderstood or incorrectly addressed.

1. Insertions

A proper safety assessment includes a molecular (genomic) level profile of the modified plant. In my original report I said that Mahyco had not eliminated the possibility that there is more than one insertion of recombinant DNA and that all insertions are not free of vector "backbone" DNA. Such confirmatory experiments are relatively inexpensive and the methods so common that they are taught in some high schools, so there should be absolutely no reason to fail on this first step of a safety assessment.

GEAC has summarised the insert data, but to my knowledge has not based this summary on any updated evidence. The following are reasons why GEAC cannot conclude from Mahyco's data that there is a single insert and no additional inserts of unexpected size or sequence composition.

A. The Southern blot analysis is fundamentally flawed and incapable of finding unexpected inserts.
    a. The probe is described as "cry1Ac probe" or "Bt" probe (p. 34)[1], presumably meaning the *cry1Ac* transgene and the probe was presumably appropriately sequence modified to reflect changes introduced by the developers[2]. ECII also refers to a single probe called "Bt" in section 3.1.4. Since the probe is specific to only this part of pMON10518, the blot is inappropriate for establishing that there are no other inserts and no backbone DNA from pMON10518. This is the scientific equivalent of using a microscope to track asteroids.

[1] Page numbers are from document called Toxicology and allergenicity studies vol. 1.
[2] "Both regions of the cry1Ac gene were genetically modified for increased plant expression using a strategy comparable to that described by Perlak…" (p. 33).

Later in ECII, GEAC asserts that Mahyco has used "the entire pMON10518 plasmid as a probe, as well as the nptII gene and 7S terminator-right border regions as probes. No additional bands were detected using these probes, indicating that there are no additional fragments from the construct at other locations in the genome." *However, I could not find these data either in the materials release in 2008 or in late 2009. Where are these data? In addition, the GEAC summary does not address the important point that there are no sensitivity parameters upon which to build confidence that any unexpected or unanticipated insertions would be detected (see below). The conclusions of GEAC on this point are based on the strength of a negative result (non-detection), which could have multiple causes besides the absence of additional inserts.*

b. As recommended by Codex Alimentarius, Mahyco should disclose all details that are necessary to establish the sensitivity of their analyses. Therefore, the size of the probe and the stringency of the wash procedure should be reported. All probes should be shown on the plasmid map. Partial fragments of transgenes or genomic DNA interspersed into transgenes have been detected as fragments as small as 15 bp[3]. Mahyco would have to show that all its probes would have detected such small insertions at the sensitivity of 0.5 copies per diploid genome in order to establish with reasonable certainty that the negative result was meaningful.

   It is impossible to determine the sensitivity of the methods used to attempt to detect unexpected or unanticipated additional inserts from the documents provided.

Note that Codex makes specific recommendations on molecular characterization: The sensitivity of all analytical methods should be documented (p. 11). Codex, (2003). Codex Work on Foods Derived from Biotechnology. CAC/GL 45-2003.

c. The sensitivity of genomic profiling methods (e.g. Southern blotting) for surveying insertions of partial transgenes should be at least to the standard of

[3] https://bat.genok.org/bat/?sp=html/practical_assessment/ch2_DNA_to_insert/molecular_characterization/southern_blots.html

published studies[4]. A combination of FISH, fiber-FISH and Southern analysis are used to increase detection of unanticipated or unintended inserts, whereas Mahyco has only used Southern and PCR. These other published studies found that even to their much higher standard, they failed to detect all insertions initially. They found for example that "[t]ransgenic oat line 3830…was previously characterized with FISH, fiber-FISH, and Southern analyses and shown to have a single major transgene locus estimated to be ca. 15 kb in length. However, when T1 progeny of line 3830 were analyzed by Southern blot hybridizations with longer exposure times and more genomic DNA per lane compared to these previous analyses, two additional minor transgene loci were detected" (Makarevitch et al. 2003). Their work emphasizes how vulnerable analyses are to arbitrary exposure times, probe sizes and wash stringency.

d. The PCR data does not substitute for the required Southern data because small fragments cannot be expected to insert in the correct order or proximity to primers for easy amplification[5].

e. The Southern blot provided as evidence (p. 46) is below acceptable standard for other reasons as well. A light band of the same size can 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, and thus the use of controls that are also GM plants. Since we do not know the sensitivity of the probes, the possibility that the control lines carry a single simple insert cannot be ruled out with this data. In addition, larger bands are seen to hybridize and these could have secondary inserts to which the probe binds. The only way to resolve these possibilities is to clone and sequence all visible bands.

f. Mahyco should meet the dual standard of demonstrating comprehensive coverage in their search for pMON10518 DNA (which the list of probes does not) and demonstrating appropriate sensitivity to small inserts (which the data do not).

References

Makarevitch, I., Svitashev, S.K. & Somers, D.A. (2003). Complete sequence analysis of transgene loci from plants transformed via microprojectile bombardment. Plant Mol. Biol. 52, 421-432.

[4] https://bat.genok.org/bat/?sp=html/practical_assessment/ch2_DNA_to_insert/example.html

B. Mahyco previously argued incorrectly that there could be no vector sequences transferred and did not to my knowledge provide any evidence of having verified their assumption. GEAC has not addressed this criticism.

The claim by Mahyco was that during Ti plasmid-mediated DNA transfer from *Agrobacterium tumefaciens* to plants, only the T-DNA is transferred. These claims begin on page 32 (section 3.3). This view is clearly wrong. First, the expectation is inconsistent with the biochemical process (Waters and Guiney, 1993). Second, at least since the mid-1990s it has been known that the "long transfer – the collinear transfer of DNA past the traditional left border – is a common phenomenon" (Wenck et al., 1997).

"In this study, we report the surprising result that approximately 75% of the transgenic plants that we generated using Agrobacterium-mediated T-DNA transfer contained binary vector 'backbone' sequences integrated into the plant genome. We obtained these data using both DNA blot and PCR analyses of the DNA of these transgenic plants" (Kononov et al., 1997, emphasis added).

Outdated views such as those expressed by Mahyco derive from previous experiments that were not designed to detect backbone transfers. *"Usually, transfer of only the non-T-DNA sequences to the plant would remain undetected because: (1) there is no selection for the transfer of such sequences; and (2) scientists generally have not looked for the transfer of these sequences"* (Kononov et al., 1997, emphasis added). Mahyco continues this tradition. The amount of DNA that can transfer can be many times the length of the T-DNA region: *"extremely long regions of DNA (greater than 200 kbp) can transfer to and integrate into the genome of plants"* (Kononov et al., 1997, emphasis added). More troubling, short backbone sequences can transfer and be difficult to detect. *"In many instances, vector 'backbone' regions of a binary vector are smaller than what is conventionally termed the 'T-DNA' region"* (Kononov et al., 1997, emphasis added).

These scientific facts invalidate additional claims made by Mahyco, notably that "The border sequence itself is not entirely transferred during the T-DNA insertion in the plant genome. This means that the inserted DNA is no longer functional T-DNA, i.e., once integrated into the plant genomic DNA, it can not be remobilized into the genome of another plant even if acted upon by vir genes again" (p. 32). Note that Mahyco has rested

[5] https://bat.genok.org/bat/?sp=html/practical_assessment/ch2_DNA_to_insert/example.html

its understanding of the T-DNA transfer process on references that are 1992 or older, and clearly no longer apply.

Mahyco also makes the claim that the disarmed Ti plasmid "does not transfer to the plant cells but remains in the Agrobacterium" (p. 32). The Ti plasmid is a conjugative plasmid. It has been known since the 1980s that conjugative plasmids transfer to eukaryotes including plants. At some frequency, the Ti plasmid itself may transfer to the plants (Buchanan-Wollaston et al., 1987, Ferguson and Heinemann, 2002, Heinemann, 1991). It is Mahyco's burden to demonstrate with proper molecular analyses that this did not happen. No data are presented by Mahyco to establish the absence of Ti DNA, *and this is not denied by GEAC*.

Finally, there is no indication of a left border on the pMON10518 map (p. 40) or on the list of genetic elements (Table 3.2, p.48). If the left border is indeed missing, then the entire plasmid will transfer.

GEAC should be aware of these facts and should have, in my view, required the company to properly demonstrate that no unanticipated or unexpected additional inserts exist. India has several reasons to be concerned by the possibility that additional sequences exist. First, there is the biological rationale for characterizing all inserts as part of proper hazard identification. Second, all inserts constitute new "events", and events may be patented. This second reason has important trade implications. If sequences common in backbones are described as the intellectual property of others in importing countries, then India may lose its control of domestically-produced brinjal.

In its review of the molecular characterization (ECII section III), GEAC fails to indicate that it is aware of the deficiencies in Mahyco's experiments, and commits its own errors of logic. For example, GEAC asserts: "*Agrobacterium*-mediated transformation has been used for the development of numerous biotech crops grown around the world for the past two decades, and has a proven track (*sic*) from a biosafety standpoint."

GEAC is wrong to associate its views on the safety of some commercialized products with the conclusion that the method of development has a history of safe use. There are a limited number of commercialized products derived from *Agrobacterium*-mediated transformation and these products are considered safe by competent authorities in some countries based on both a pre-market assessment and their use in structured agricultural systems. The number of products being used is still very small and does not warrant an

endorsement of the safety of a particular procedure. For example, considering the number and the kinds of products based on nuclear fission (from bombs to power plants) it could be asserted that in the context in which products of nuclear fission are deployed these products have a safe track record. This does not mean that every nuclear fission reaction is inherently safe and by extension every bomb or powerplant in every context is inherently safe just because it is based on nuclear fission.

References

Buchanan-Wollaston, V., Passiatore, J. E. and Cannon, F. (1987). The mob and oriT mobilization functions of a bacterial plasmid promote its transfer to plants. Nature 328, 172-175.

Ferguson, G. C. and Heinemann, J. A. (2002). Recent history of trans-kingdom conjugation. In Horizontal Gene Transfer, M. Syvanen, and C. I. Kado, eds. (London and San Diego, Academic Press), pp. 3-17.

Heinemann, J. A. (1991). Genetics of gene transfer between species. Trends Genet. 7, 181-185.

Kononov, M. E., Bassuner, B. and Gelvin, S. B. (1997). Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration. Pl. J. 11, 945-957.

Waters, V. L. and Guiney, D. G. (1993). Processes at the nick region link conjugation, T-DNA transfer and rolling circle replication. Mol. Microbiol. 9, 1123-1130.

Wenck, A., Czakó, M., Kanevski, I. and Marton, L. (1997). Frequeunt collinear long transfer of DNA inclusive of the whole binary vector during Agrobacterium-mediated transformation. Pl. Mol. Biol. 34, 913-922.

2. Expression: Novel RNA and Proteins

As indicated in my previous submission, Mayhco has not provided information on potential novel RNAs and proteins produced in the six possible open reading frames created by the EE-I event or by undetected secondary insertions. In fact, Mahyco has provided no information whatsoever on novel RNAs. This is a significant omission. Moreover, since Mahyco uses the nos3′ terminator in its construct it has an added obligation to look for novel RNAs. The nos3′ is not an efficient terminator in eukaryotes, leading to read-through, longer mRNA molecules and potential fusion proteins.

According to Codex (p. 14, paragraph 32), "Information should be provided on any expressed substances in the recombinant-DNA plant; this should include: the gene product(s) (e.g. a protein or an untranslated RNA); the gene product(s)' function…".

Codex, (2003). Codex Work on Foods Derived from Biotechnology. CAC/GL 45-2003.

In response to my previous submission, GEAC (ECII) has commented that "the insertion sites from Bt brinjal event EE-1 was isolated and sequenced. The 3' end of the *cry1Ac* gene was examined and found to have the expected stop codon, followed by the 7S terminator and right border. Genomic flanking sequence examined on either side of the insertion showed no significant matches [to] any sequence in GenBank, and does not encode any open reading frames in all six frames. This suggests that no novel RNAs or proteins were generated as a result of the EE-1 insertion."

A. This response is based on assumption, not evidence. Moreover, the assumptions are not reliable.

    a. I indicated in my previous submission that the presence of stop codons and terminator sequences are not sufficient to prove the absence of unintended or unanticipated RNA and protein products.

    For example, Mahyco has used the nos3′ terminator adjacent to the nptII gene. The nos3′ is not an efficient terminator in eukaryotes, leading to read-through, longer mRNA molecules and potential fusion proteins (Rang et al., 2005).

    Researchers first reported this in a transgenic soybean called 40-3-2. Interestingly, 40-3-2 is a Monsanto product and Monsanto is presumably the source of pMON10581. Even more troubling, "the read-through transcript [in 40-3-2] was processed in four different RNA-variants" (Rang et al., 2005). The variants might arise from splicing pathways or other pathways that are not predicted from Genbank DNA sequences.

    A significant concern raised by the authors about the variant RNA molecules in 40-3-2, if they arise through a splicing mechanism, was that the nos sequence itself harbors a splice site. "The cis regulatory regions that initiate and mediate splicing are located within the removed region of spliced transcripts. If this is also true for the mechanisms mediating posttranscriptional processing of the described variants, it seems reasonable

to assume that the transcribed nos terminator region might be responsible for processing the RNA. *Since the nos terminator was and still is commonly used as regulatory region in the production of genetically modified crops, read-through products and RNA variants could also be expressed in these plants*" (Rang et al., 2005, emphasis added). Since nos is also used in Bt binjal, these established research findings require that it be evaluated for variant RNA species arising from the inserted DNA.

This research has been extended by others generalizing to other genetically engineered plants based upon Monsanto-derived vectors and transgenes.

> The experiments showed the synthesis, due to the known loss of the NOS terminator, of fusion RNA variants coding for putative CRY fusion protein showing 2 or 18 putative additional aminoacids, composed of the 3′ end of truncated cry1A(b) gene and the putative HECT 3′ sequences transcribed in antisense orientation. Different transcripts showed a series of deletions some of which in common to all observed RNAs…In silico translation of putative fusion RNAs did not show significant identities with known protein domains…Taken together, our data, while suggesting the insertion of the transgenic sequence in a putative HECT gene, *show the transcription of new fusion RNAs, a result similar to that obtained by Rang and et al. (2005) in Roundup Ready Soybean 40-3-2* (p. 280 Rosati, A. et al., 2008, emphasis added).

Note that these novel variants were not predicted from Genbank analyses of open reading frames. This is emerging as a general theme in molecular biology and cannot be ignored by the industry or the regulator. In addition to transcriptome changes, there are unpredicted changes in the proteome. For example:

> A comparison of a commercial maize variety carrying the MON810 event [from Monsanto] and its isogenic relatives (Zolla, L. et al., 2008) indicates both that unanticipated changes occur as a result of the engineering process and that these can be more carefully characterized using profiling techniques that are not common in the scientific dossiers provided to regulators (Heinemann, J.A., 2007).
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> The commercial line (33P67) was confirmed by Southern blotting to have a single insert (Zolla, L. et al., 2008). The subsequent analysis was on the proteome,

comparing seeds of the commercial line and the seeds of its immediate F1 generation (33P67F1) with the seeds of the isogenic comparator (33P66) and the seeds of its immediate F1 generation (33P66F1). These comparisons allowed the researchers to measure variability in the proteome that was caused by the environment (33P66 vs. 33P66F1 and 33P67 vs. 33P67F1), effects caused only by the engineering process (33P66F1 vs. 33P67F1), and the combined effects of environment and engineering (33P66 vs. 33P67).

Approximately 100 of the identifiable proteins varied either quantitatively (increases or decreases but protein in both) or qualitatively (protein present only in one) in the comparison of the seeds from the conventional line and the seeds it produced (33P66F1). Similar numbers of statistically significant changes were observed in the seeds of the transgenic 33P67 and its F1 progeny (78 changes). This is the effect of breeding and the environment on proteome changes because the tested seeds (33P66 and 33P67) came from plants grown in different environments. Comparisons between 33P66 and 33P67 reveal the combination of differences in environment and effects of the genetic engineering process. There were 27 statistically significant proteome differences between the conventional and transgenic seeds.

This illustrates that only thorough scientific testing, and not assumption-based reasoning of the kind used in the ECII, can properly be used for risk assessment.

References[6]

Heinemann, J.A. (2007). Letter to the Editor. Environ Plann Law J 24, 157-160.

Zolla, L., Rinalducci, S., Antonioli, P. & Righetti, P. G. (2008). Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. J Proteome Res 7, 1850-61.

Regardless of whether the variant RNAs arise from a cryptic splice site within nos or through other processing pathways, all novel RNA species in Bt binjal must be reported for a proper safety assessment. GEAC should be adhering to the full extend of Codex Alimentarius guidelines, and not picking and choosing which standards to enforce and which to excuse.

6 https://bat.genok.org/bat/?sp=html/practical_assessment/ch2_DNA_to_insert/breeding_trees_equivalence.html

References

Rang, A., Linke, B. & Jansen, B. (2005). Detection of RNA variants transcribedfrom the transgene in Roundup Ready soybean. Eur. Food Res. Technol. 220, 438–443.

Rosati, A., Bogani, P., Santarlasci, A. & Buiatti, M. (2008). Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize. Plant Mol. Biol. 67, 271-81.

b. The failure to adequately assess the kinds of novel RNAs produced at the site of insertion is compounded by the very real possibility that there exist additional uncharacterized and unanticipated insertions, each of which could be responsible for generating unknown and unanticipated novel RNA and proteins. Two kinds of experiments would put this question to rest:

   i. proper and full profiling of the genome by a combination of techniques (done properly, as discussed above); and
   ii. transcriptome[7], proteome and metabolome[8] profiling:

> Non-targeted, analytical approaches at the gene, transcript, protein and metabolite levels are the methods-of-choice for investigating the physiology of the GM plants as comprehensively as possible, thus increasing the chances of detecting unintended effects.
>
> Rischer, H. & Oksman-Caldentey, K.-M. (2006). Unintended effects in genetically modified crops: revealed by metabolomics? Trends Biotechnol. 24, 102-104.

Disappointingly, GEAC (ECII) dismissed Dr. P.M. Bhargava's previous recommendation for profiling for the following reasons:

- the techniques are expensive and have "little value"; and
- they are not validated.

These assertions are not based on evidence and are not in my opinion—and that of other biosafety experts—correct[9]. In fact, even Mahyco's

[7] https://bat.genok.org/bat/?sp=html/topic_guides/ch1_basics/profiling_hazards/molecular_methods/transcriptome_techniques/main.html

[8] https://bat.genok.org/bat/?sp=html/topic_guides/ch1_basics/profiling_hazards/molecular_methods/proteome_techniques/main.html

collaborator, Monsanto, has demonstrated its ability to perform such profiling.

For example: "[T]his method [2D gel electrophoresis] could be used to interrogate proteome alterations such as a novel protein, fusion protein, or any other change that affects molecular mass, isoelectric point, and/or quantity of a protein" (p. 2154).

Monsanto study published under Ruebelt, M. C., Leimgruber, N. K., Lipp, M., Reynolds, T. L., Nemeth, M. A., Astwood, J. D., Engel, K. H. & Jany, K. D. (2006). Application of two-dimensional gel electrophoresis to interrogate alterations in the proteome of genetically modified crops. 1. Assessing analytical validation. J. Agric. Food Chem. 54, 2154-2161.

- GEAC and all other regulators do accept profiling evidence. Southern blotting (done properly) to determine if unanticipated or unintended additional insertions were made during transformation is a profiling of the genome. This procedure has not been "validated", but is ubiquitously accepted.
- Mahyco's collaborator, Monsanto, can and does profile both transcriptomes and proteomes. These procedures have not been cost prohibitive for the industry, are rapidly becoming less expensive and do provide useful information. For example:

[9] see quote above by Rischer et al. and https://bat.genok.org/bat/?sp=html/topic_guides/ch1_basics/profiling_hazards/main.html

Transcriptome

Monsanto researchers conducted a survey of small RNAs found in soybean seeds, corn kernels, and rice grains (Ivashuta, S.I. et al., 2008). Although this survey was incomplete, it represents the largest such survey that I am aware of to date. These RNAs were ≤30 nucleotides long. The RNA molecules isolated from rice were sequenced using high throughput 454 procedures, for a total of 285,864 unique and sequenced RNA molecules.

The researchers were able to quantify the amount of RNA in size range of ≤30 nucleotides for mature soybean and corn seeds. Soybean had approximately 0.70 g of small RNA/gram of tissue, and corn and rice reportedly had similar amounts.

This study demonstrates that it is within the capacity of developers to perform profiling experiments for the purpose of hazard identification. The number of small RNAs reported in this study would likely exceed the number of anticipated and unintended small RNAs generated as a result of the engineering process and insertions, and thus the exercise would be even simpler when applied to the GMO.

References: Monsanto study published under Ivashuta, S.I., Petrick, J.S., Heisel, S.E., Zhang, Y., Guo, L., Reynolds, T. L., Rice, J.F., Allen, E. & Roberts, J.K. (2009). Endogenous small RNAs in grain: semi-quantification and sequence homology to human and animal genes. Food Chem Toxicol 47:353-360.

Proteome

This study demonstrated that 2DE [2D gel electrophoresis] can be utilized to reliably analyze the seed proteome of transgenic *A. thaliana* (p. 2176).

Quote from a Monsanto publication under Ruebelt, M. C., Lipp, M., Reynolds, T. L., Schmuke, J. J., Astwood, J. D., DellaPenna, D., Engel, K. H. & Jany, K. D. (2006). Application of two-dimensional gel electrophoresis to interrogate alterations in the proteome of gentically

modified crops. 3. Assessing unintended effects. J. Agric. Food Chem. 54, 2169-2177.

3. Comparator[10]

All scientific studies that form part of a safety evaluation must involve a comparator. The comparator must be appropriate and used consistently. The purpose of the comparator is to provide the standard baseline for all measurements, and be the single common element in all experiments using material grown in multiple locations and years. It is impossible to determine if either of these rules were followed in the dossier for Bt brinjal.

The Minister is or should be aware that failure to adhere to these simple scientific rules has resulted in withdrawal of other commercial products. Monsanto's dossier for LY038 and LY038 x MON810, two GM corn varieties, also failed to follow these rules. Despite LY038 being approved by several jurisdictions, both Food Standards Australia New Zealand (FSANZ) and EFSA required Monsanto to redo crucial experiments because Monsanto did not use the proper comparator. While some revised experiments were submitted to FSANZ and LY038 was ultimately approved by them, in early 2009 EFSA required other experiments to be redone. Despite an estimated US$1 billion investment in LY038 and its derivatives' marketing, processing and development, Monsanto instead chose to withdraw the product from further evaluation by EFSA and discontinued all plans for commercial production. This story illustrates the critical importance of the comparator to all the scientific findings. Getting this wrong can kill the product in other jurisdictions and for good reason: using different and/or inappropriate comparators in the many different experiments takes away the ability to identify true hazards and assess them.

The comparator is defined by Codex Alimentarius as the conventional counterpart derived from the non-GM parent.

[It] is recognized that for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts.

Codex, (2003). Codex Work on Foods Derived from Biotechnology. CAC/GL 45-2003.
Codex, (2008). Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals. CAC/GL 68-2008.

[10] https://bat.genok.org/bat/?sp=html/topic_guides/ch2_dna_to_insert/breeding_trees.html

EFSA requires that:

> The appropriate comparators have all traits in common except for the newly introduced ones.
>
> EFSA (2008). Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials. Food Chem. Toxicol. 46, S2-S70.
>
> In the case of vegetatively propagated crops, comparative analyses should include the non-genetically modified isogenic variety used to generate the transgenic lines. In the case of crops that reproduce sexually, comparators would include appropriate information required in applications for GM plants and/or derived food and feed non-GM lines of comparable genetic background.
>
> EFSA, (2006). Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. EFSA J. 99, 1-100.

While Codex and EFSA do not preclude the use of control lines in addition to the proper comparator, these additional lines should not substitute for the comparator.

What was the comparator for Bt brinjal? Was it the non-GM parent, closely related (e.g. ≥50%)? Was a single comparator consistently used in all molecular, toxicological, immunological, feeding and compositional studies? I could find little or no information on the comparator in any experiment that I reviewed. The GEAC report (ECII) mentions the comparator zero times. GEAC does mention the "conventional counterpart", but does not indicate that this was a single closely related parent used consistently in all experiments, nor does GEAC provide any details on the genotype or history of this conventional counterpart. Hence, I have low confidence that the simple, but critical, scientific practice of using the same and the appropriate comparator has been followed.

In my opinion, the dossier and the subsequent GEAC analysis (ECII) fail to meet fundamental and even routine hazard assessment standards for molecular characterization. Since this is the starting point of any risk assessment, the downstream effects on the analysis can be significant. Many of the analyses that were undertaken seem to have been half-efforts, with shortcuts taken and then retrospectively justified using non-validated or incorrect assumptions. If indeed Bt

brinjal is safe for human consumption, safe for environmental release, and the right technology for India's food security and trading future, then the certainty of this can be demonstrated using existing, affordable and effective scientific analyses.

I urge the Minister to insist on the proper standards being met so that he can be sure that the product matches the claims. A checklist of tests is provided by the Biosafety Assessment Tool (https://bat.genok.org/bat/) which is freely available to all. This checklist allows a careful regulator to assure the Minister that the science has been done to high and appropriate standards and that the guidelines set by Codex Alimentarius and competent food safety agencies have been fully addressed by the data.

With best wishes,

Prof. Jack Heinemann
30 December 2009