**COMMENTS OF DR P M BHARGAVA ON THE REPORT OF THE EXPERT COMMITTEE (EC-II) ON BT BRINJAL EVENT II-I DEVELOPED BY M/S MAHYCO**

## I. General comments

(1) Any statement that the study was approved by RCGM/GEAC or was done according to any national or international protocol is, in a scientific evaluation, irrelevant. The only question that needs to be answered is whether the study is scientifically sound and valid. In fact, many of the comments that follow are an aspersion on the approval process adopted by our approval mechanism. We should not ignore the fact that the vast portion of GMOs (84% in 2008) in use are confined to four countries (USA, Canada, Argentina and Brazil) and that some 90 percent of the member countries of United Nations have not approved the planting of GMOs or their use as food material without labelling. For inadequacies of the U.S. system of approval of GMOs, see W. Freese & D Schubert, Safety testing and regulation of genetically engineered foods. Biotechnology and Genetic Engineering Reviews, 2004, **21,** 299-324 (96 references).

(2) The toxicity tests/biosafety tests that have been done either by Mahyco (I will, for obvious reasons, use the term Monsanto, in place of Mahyco in what follows) or by private labs or by Government labs. Examples of tests done by Monsanto are on page 1 (line 14 from bottom), page 13 (lines 1-10), page 17, pages 25-29, page 46 [Section 3.3.3(1)], page 50 (4.1). Monsanto has strong vested interests, and it has an extremely derogatory record in respect of honesty, integrity, and following the law. Examples are given in Annexure 1. Monsanto also knows very well that whatever they say would be accepted by RCGM/GEAC as we have ensured that there is no organized and reliable system with high public credibility to check on its results. In view of this, tests done by Monsanto cannot be relied upon. As regards, the tests done by private or Government laboratories, the samples were given by Monsanto. I am not aware of any foolproof record available to show that, in every case, all these samples tested were the right samples.

(3) The differences found between normal and Bt brinjal have been attributed to variation in the normal (non-Bt) product. Any statistician will tell you that if the variation in the control is so large, the number of samples in both the control and the experimental groups has to be much larger that has been used. Examples of such differences are on pages 68-71, items 4, 5, 6; and pages 74-75.

(4) Before the environmental release of Bt brinjal, we should determine if we need it. Therefore, its socio-economic survey should be done before it is released.

(5) The ICAR has developed integrated pest management and the use of biopesticides against pest attack on 85 crops, including cotton and brinjal. IPM is a part of the country's agriculture policy approved by the Indian Parliament in 2001. There is already a substantial amount of data (Annexure 2) which clearly establishes that IPM and biopesticides work just as well (if not better) than the Bt gene in the case of brinjal. Why is it then that ICAR and Agriculture Universities are not propagating the use of IPM and biopesticides? Is it to please and favour Monsanto?

(6) Many studies (for example, on page 13 and page 38) have been done with surrogate protein. No data is provided on the surrogate protein. Is it the real Cry1Ac protein? Or is it the chimeric protein used in this study? Was the surrogate protein sequenced? If so, where is the sequence given? In any case, for these studies to be meaningful, they should have been done with defined plant extracts containing Bt protein. (The report mentions that the surrogate protein is "similar" to the protein in plants; "similar" does not mean "identical".)

(7) I am enclosing (Annexure 3) a commentary I wrote in July 2008 in reply to an article in *Nature Reviews Genetics* which is, perhaps, one of the best defences of DMOs and critique of opposition to them by NGOs. This fully referenced commentary not only explodes the myths that are flouted regularly regarding opposition to GMOs, but also gives references to the following adverse effects of GMOs:

- altered structure and immunogenicity of a transgene when expressed in the host.
- adverse effect on soil ecology
- reproductive interference
- propensity to cause cancer
- adverse effect on non-targetted organisms
- development of resistance

## II. Specific comments

(i) Page 12 (food cooking and protein estimations): According to the data provided, even non-Bt brinjal that was not cooked, scored positive for Bt protein! This makes the full set of data suspicious. Further, the sensitivity of the method to detect Bt protein is not given.

(ii) Page 17: Why only acute or sub-chronic toxicity studies? Why not long-term toxicity studies in rats/mice/rabbits which are perfectly possible and done for drugs? The reasons given for not doing such studies on page 59 and 87, are not scientifically valid. A large number of proteins are known to lead to cancer when mutated (examples: *ras*, *myc*, oncogenic viral proteins). Further, toxicity studies should be done with defined plant extracts containing the Bt protein in plant, and not on a surrogate protein.

(iii) Page 35: India has the largest number of vegetables (over 150) in use, with a vast variety in many of them. Our eventual *potential* for export of our vegetables, many of which have important pharmacological action, is enormous and could easily run into a

hundred thousand crores a year. We *can* capture, say, three quarters of the world vegetable market. All this market will be lost if we allow GM vegetables. Eighty four percent of our farmers are small or marginal farmers with a holding of less than 4 hectacres. According to Monsanto's own data, Bt brinjal pollen can travel for 30 metres and could thus easily contaminate the neighbouring non-Bt brinjal field. In course of time, we would be left with no non-Bt brinjal population even if the farmers do not want Bt Brinjal. Unlike in Europe, Britain and many other countries, we have no labelling laws. In these countries, any food product which has more than 0.9 percent of GM material, must be labelled as genetically modified. Therefore, neither will we be able to export our vegetables nor will *we* be able to exercise choice in regard to GM brinjal or non-GM brinjal. Just extend it to all vegetables and imagine the consequences. There is an ever-increasing demand everywhere, including in our country, of organically grown food which fetches the farmer better price. This market will also be lost.

(iv) Page 36: The comments on "gene transfer from brinjal to other plants" or"gene transfer from brinjal to other organisms" are totally invalid. There is an enormous amount of evidence (Annexure 4) of horizontal gene transfer across species. It is believed widely that more than 10 percent of all the genes in all living organisms are a consequence of horizontal gene transfer. Species non-specific viruses are known; they become non-specific on account of high mutation rate. A Nobel Prize was given to Joshua Lederberg for discovering the process of transduction in which viruses carry a gene from one organism to another.

(v) Page 41-42, item 3.2.4: Who did these studies on possible accumulation and persistence of Bt protein in the soil? Was it Mahyco/Monsanto? The half-life of Cry1Ac protein is reported by EC-II to be 9.3 to 40 days in soil (where ? in India?). These levels are not low. A statement that no Bt protein was detected in any of the soil samples goes against the above-mentioned half-life.

(vi) Page 43, Section 3.3.1 (toxicity and allergenicity of pure proteins): What is reported here is invalid, as pure (probably surrogate) protein and not plant extract containing the protein product in the plant was used.

(vii) Page 46 (alkaloid content): The samples given by Monsanto were not checked by IICT as regards their authenticity. The actual data does not support the statement made in the report that there was no significant difference between the alkaloid content of Bt and non-Bt brinjal.

(viii) Pages 54, 55 (Issue 1): It is a misnomer to call the gene inserted in brinjal as Cry1Ac, as it was a chimeric protein. Further, the two statements made here, "this gene is 99.4 percent identical to native Cry1Ac" and "this difference of 0.6 percent is attributed to the difference in presence of one amino acid at position 766, that is serine in place of leucine", are contradictory. If the difference is 0.6 percent and the protein construct has 1178 amino acids, the difference has to be of 6 or 7 amino acids. In that case, it is absolutely incorrect to call it Cry1Ac protein. And difference of one amino acid *can* change everything, what to say of 6.

(ix) Page 55 (Issue 2): Why was *aad* gene which confers streptomycin resistance, used? It can surely cause undesirable effects.

(x) Page 57 (Last para): See comments under item (v).

(xi) No studies have been done on change in the soil bacterial species.

(xii) No studies have been done also on the effect of Bt brinjal on soil micro-nutrients.

(xiii) Page 85 (Item 1), and page 86 (Items 4,5): Introduction of a transgene is well-known to increase the number of mutations, in comparison to normal breeding [A.K. Wilson et al., Transformation-induced mutations in transgenic plants: Analyses and biosafety implications, Biotechnology and Genetic Engineering Reviews, 2006 (Dec.), **23**, 209-236; National Academy of Sciences (2007), "Safety of genetically engineered foods. Approaches to assessing unintended health effects". Report of "Committee on identifying and assessing unintended effects of genetically engineered foods on human health", Institute of Medicine and National Research Council, National Academy of Sciences, Figure 3.1, pp.64-65]. There can also be insertion of small transgene fragments at various sites which would not be detected by the probes used for detection of transgene. These insertions can lead to interference in function of other genes which, in turn, can lead to changes in protein, RNA and metabolite make-up which, together, play an important role in determining the function of a living organism. That is why DNA fingerprinting, proteomics, transcriptomics (for an application of transcriptomics revealing multiple modulatory effects of a plant-based drug, see *FEBS J.***,** 2009, **276**, 1450) and metabolomics (for an application of metabolomics, see BBRC, 2009, **386**, 268) studies have become essential. To say that techniques such as proteomics "are currently in their infancy" is, to say the least, absurd. There are several highly cited journals, listed in Current Contents, that are totally devoted to proteomics, and there are commercial companies even in India, doing proteomics studies on order. Two references that support the above view, are:

(a) M. Malatesta *et al*., A long-term study on female mice fed on a GM soyabean: effects on liver ageing, Histochem Cell. Biol. 2008. **130**, 966, in which GM soyabean fed mice showed a more marked expression of liver senescence markers (as determined by proteomics);

(b) L. Zolla *et al*., Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modification, Journal of Proteomic Research, 2008, **7**, 1850-1861.

(xiv) Page 86 (Item 3): What about changes in the glycosylation pattern of other proteins? Proteomics will tell you that.

(xv) Page 86 (Item 6): Environment is of course important. But the fact is that surface properties that are genetically determined are more important. The capability of an organism is determined by its genetic make-up, while environment determines the extent to which these capabilities would be converted into abilities. Ignorance of this rule can be disastrous.

(xvi) Page 86 (Item 7): By reproductive interference, I mean reduction in the reproductive ability of animals which has been demonstrated with GM food crops. (For reference, see Annexure 3)

(xvii) Page 86 (Items 8, 9): See item (iii) above.

(xviii) Page 87 (Item 10): These techniques need to be developed *before* environmental release so that we have a method of detecting contamination at the level of 0.01 percent.

(xix) Page 87 (Item 11): Unless experiments have been done, one cannot arrive at any conclusions.

(xx) Page 88 (Item 13): Please see item (ii).

(xxi) Page 87 (Item 12): The answer given is totally inadequate and irrelevant. The question is not what X, Y or Z says should be done. The world's entire scientific wisdom does not lie with them, especially as they have vested interests. The question is what is scientifically valid and logical. What I have said should be done is so obvious! Why don't we take lessons from the process of release of drugs?

(xxii) Page 88 (Item 14): I would like to have details of studies looking at effect of Bt brinjal or Bt-anything on cattle micro-flora. The other studies referred to are not relevant. The question is not whether or not composition of the diet influences micro-flora. The question is, does the presence of the toxic Bt gene and all other possible changes in the plant (which only proteomics, transcriptomics and metabolomics will reveal) influence the microflora? All the evidence points towards the probability that the death of several thousand cattles in Warangal District over a period of two years was on account of their consuming Bt cotton plant remnants. The intestines of these animals were found shrivelled. This could be a consequence of Bt toxin having an adverse effect on the rumen microflora and thus on digestion. This clearly needs to be studied. Why is there such a reluctance to do such studies? Is the company afraid that such studies done in an unbiased way, will go against the company?

(xxiii) Page 89 (Items 16 and 17): The question is not answered. We are talking of Bt brinjal, not Bt cotton. No part of Bt cotton plants is consumed by humans – not even in a famine!

(xxiv) Page 89 (Item 18): Who did these studies and where have the results been published? Can I have a reference and the details of these studies?

(xxv) Page 89 (Item20): Who did these studies and where are the results? Can I have a copy?

(xxvi) Page 90 (Item 21): Three years is too short a period. Many examples can be given (for example, in the area of reproduction).

(xxvii) Page 90 (Item 22): How would you determine translocations which can even lead to cancer, if you do not do karyotyping? With automated machines widely available, this is one of the simplest experiments to do for a biologist.

(xxviii) Page 90 (Items 23, 24): The question is: given the same required *minimal* inputs, is the productivity (biomass and yield of the desired product) the same, better or worse.

(xxix) Page 90 (Item 25): If you don't know ecology how would you know what are the non-target organisms in the area?

(P M Bhargava)
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