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A review of Mahyco's GM Brinjal food safety studies

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Introduction

This document has been written in response to Mahyco's dossier in the public domain and a request for an appraisal of the data by lead Petitioner in the Supreme Court of India, Aruna Rodrigues.

The Institute of Health and Environmental Research Inc. (IHER) is a not-for-profit research institute with an interest in genetically modified (GM) organisms, particularly those destined for food. Its directors hold the following degrees: ordinary degrees in Medicine, Science and Agriculture, Honours Degrees in Agricultural Science and Organic Chemistry, a Master of Public Health, and PhDs in Plant Genetics and Medicine. The Directors have training and expertise in plant science, agriculture, medicine, chemistry, biochemistry, nutrition, animal feeding studies, epidemiology and biostatistics. IHER has received funding from the government of Western Australia to conduct one of the first long-term, independent animal feeding studies ever done into the safety of GM crops measuring human health end-points. IHER receives no funding from biotechnology interests and hence is completely independent of them.

Dr Judy Carman BSc (Hons) PhD MPH MPHAA is a Director of IHER and the author of this document. She:

- Has a science degree, an Honours degree in organic chemistry, a PhD in medicine in the field of metabolic regulation and nutritional biochemistry, specialising in animal feeding studies, and a Master of Public Health specialising in epidemiology and biostatistics.
- Worked in the fields of human nutrition (including at the CSIRO), infectious diseases including HIV/AIDS, national injury surveillance and analysing data from Divisions of General Practice.
- Was the Senior Epidemiologist in the Communicable Disease Control Branch of the South Australian Department of Health, investigating outbreaks of disease in the state including food-borne, mosquito-borne (eg Ross River Virus), zoonotic and pneumonia from *Legionella*. She was Acting Head of the Branch at times.
- Taught at an agricultural college and two Australian universities to the level of Senior Lecturer.
- Was Assistant Director and Senior Lecturer, Research Centre for Injury Studies (RCIS), Flinders University of SA, incorporating the National Injury Surveillance Unit (NISU) of the Australian Institute of Health and Welfare (AIHW).
- Held a number of positions in the Public Health Association of Australia (PHAA), including being spokesperson on GM foods and convening two national food conferences for the PHAA.
- Has analysed various State and Federal government disease, general practitioner, hospitalization and death surveillance systems, and have written several official State and Federal government reports from these systems, on eg cancer, communicable diseases and various injuries.
- Has advised or been consultant for parliamentarians, government and non-government organisations and industry bodies on various matters.
- Was consultant on GM foods to the Hon. Kim Chance, Minister for Agriculture and Food in Western Australia.

- Has presented to the New Zealand Royal Commission into Genetic Modification.

Review

This review concentrates on the food safety evaluation done by Mahyco as reported in Chapter 7 of Volume 1 of their submission and critiques that section.

Compositional comparisons

Once of the greatest concerns about the process of genetic engineering is that the actual process of inserting the gene may cause the plant to up-regulate or down-regulate the normal genetic expression of the plant and hence to produce more of something harmful to human health, or less of something beneficial to human health. An associated concern is that the insertion process may cause the plant to produce a novel substance for that plant. There are certainly examples of all of these effects appearing as a result of genetic engineering. Yet, the compositional analyses presented to the Indian Government by Mahyco do not assess these known likelihoods.

For example, the compositional comparisons concentrate on measuring moisture, protein, oil, ash, carbohydrates, calories for fruit tissue, nitrogen, ash and crude fibre. These are extremely crude measures of the nutritional components of brinjal. A full protein analysis would have gone some way to determine if the plant was producing more, or less, of something, or a completely new substance. Yet it was not done.

Moreover, according to page 104 of Mahyco's document, a sample size of only three Bt brinjal and three non-BT brinjal were used to determine the differences in composition between the GM and non-GM brinjal. This is woefully inadequate to determine compositional differences between two crops. The composition of the two crops (the 'clinical' difference) would have to be profoundly different to be able to be picked up as a statistical difference using such a tiny sample size. Also, the only real way of comparing the composition in this manner is to grow the GM and non-GM parent brinjal from which the GM brinjal was developed, side-by-side in the same field, under the same conditions of soil type, fertilizer, herbicides, insecticides, water, sunshine, etc, and then to use samples from these plants in the comparison studies. Only then can any differences between the GM and non-GM crops be determined to be due to the genetic insert and not due to confounders such as soil type, fertilizer, herbicides, insecticides, water, sunshine, etc. Yet Mahyco do not describe if their samples were obtained in this manner or not.

The analyses presented also do not take into account compositional differences found under different growing conditions in different areas of India. For example, no work seems to have been done on whether the concentration of harmful components of Bt brinjal increase under different climatic conditions, eg heat or water stress. In order to do this, the comparative growing study described above, where GM and non-GM parent brinjal are grown in the same field under identical conditions, would need to be repeated in various places in India under

different climatic and soil conditions. These do not appear to have been done.

In addition, as woefully inadequate as simple amino acid and fatty acid profiles are, even these do not appear to have been done by Mahyco. (Amino acids are the building blocks of proteins, while fatty acids are the components of fats.)

Moreover, information about the chemical composition and alkaloid content measurements did not provide the following standard and required statistical information: the mean and standard deviation of each group, the nature of the statistical test done and the p-value resulting from the statistical test. Furthermore, the analysis of alkaloid content in GM brinjal does not even provide information as to how many brinjal were tested in each group. For the Cry1Ac protein estimation in brinjal after cooking, no cooking temperatures or samples sizes were given. Mahyco also appears not to have undertaken any studies to determine if the GM DNA in GM brinjal can degrade upon cooking.

GM crops are deemed to be substantially equivalent to non-GM crops until they fail some type of substantial equivalence test. Yet no decision has been made as to what this test should be; how compositionally different a GM crop needs to be from a non-GM crop to be regarded as different. To elaborate, if there had been a decision made that a GM crop is judged to be compositionally different if say 10% of its amino acids are statistically significantly different when fruit from 50 different brinjal plants are measured, or that a full protein analysis needs to be done and the GM brinjal needs to have all proteins within 10% of the levels present in non-GM brinjal, then there would be a clear hurdle that GM brinjal would need to clear to be deemed to be substantially equivalent. But there is no such hurdle. Instead, there is a bland statement by the producers of GM brinjal that their crop is substantially equivalent without even describing the scientific criteria they have used to determine substantial equivalence or any pass/fail level they may have within these criteria.

Many of the errors described constitute errors of research methodology which can only be corrected by conducting appropriately-planned and executed research. Until this work is done, it cannot be stated that the composition of Bt brinjal is similar to ordinary brinjal.

In summary, the information submitted by Mahyco is completely inadequate to determine if the composition of Bt brinjal is similar or different to ordinary brinjal. Moreover, the information presented do not meet accepted scientific standards of reporting.

Allergy assessments

To determine allergenicity of the Bt brinjal, Mahyco first did a paper-based analysis. It artificially split the GM protein that it expected to be produced (not any unexpected proteins) into smaller segments and compared the segments to certain data bases of known allergens. It should be remembered that not all allergens are known, even in peanuts, and that, even for the known allergens, not all are represented in these databases.

Mahyco also reported a skin irritation test on rabbits and a mucous membrane test using vaginal tissue in rabbits on pages 113-116 of volume 1. For both studies, only three female rabbits were used for each treatment group and the animals followed for only 72 hours after

exposure. The studies cannot be regarded as allergy tests as the test substance was only applied once. Allergies generally require repeated exposure to a substance before an allergy can be developed. Then, the more often the exposure, the worse the allergic reaction tends to get. Although clinical signs of matters such as toxicity and skin reaction were measured in this test, there is no description as to exactly what these involved and what would constitute an adverse finding. Moreover, no matter what measurements were taken, calculations indicate that even if all rabbits treated with the GM material showed a severe adverse clinical effect compared to no rabbits suffering this effect in the non-GM-treated rabbits, the appropriate statistical test would be completely unable to find statistical significance due to the small number of animals used.

The methodology of the allergy study undertaken in Brown Norway rats does not meet the standards of allergy testing employed by other researchers that **have** found allergic reactions due to consumption of GM crops[1] and the full results were not given in the text.

Reproductive studies

Mahyco did not provide any reproductive studies and it therefore appears not to have done any, even though adverse reproductive effects have been found from eating other GM crops[2,3]. These results strongly indicate that reproductive studies should be required before any GM crop could be assessed as safe to eat.

Digestive studies

Digestive studies used an *in vitro* (in glass) method of determining how quickly the protein that is expected to be produced will break down in the intestine. No data appear to have been given for the digestibility of GM DNA. Such studies are notorious for providing false assurances about the digestibility of GM DNA and proteins. For example, such studies often use unrealistically high levels of stomach acid and digestive enzymes. The level of acid in a human stomach moves towards neutral once food enters it. The only real way to determine how quickly GM DNA and protein are digested is to do experiments in animals or humans. Several of these *in vivo* studies have shown that GM DNA can and does survive digestion and can be found in tissues of the body. A recent study in Italy found that GM DNA present in the feed of cows could even be found in milk on supermarket shelves[4].

Acute toxicity studies on animals

The results of these studies cannot be used to determine the safety of GM brinjal, as described below.

Acute toxicology test on mice

This test was not done using the GM proteins as expressed in the GM plant that people will be eating. Instead, Mahyco used proteins that were produced by GM bacteria that were

engineered to produce the GM proteins. Mahyco appears not to have determined if the proteins are exactly similar in structure and function as those found in the plant, even though it is known that the expression of the same DNA in different organisms can produce proteins with different physiological effects[1]. Moreover, the study on the Cry1Ac protein used only 10 mice per group, a seriously insufficient number to determine the true clinical outcomes of these mice, while the number of mice used to test the NPTII protein is not given. It appears that body weight and food consumption were the only real measurements taken for the Cry1Ac protein study because, while tissue samples were taken, they appear to only have been kept and not analysed. Furthermore, while pathological changes were seen in the 'gross necroscopy' in some mice, neither the nature of the necroscopy nor the nature of the changes were described. Nor were the nature of the statistical tests, the means, standard deviations and p-values of the analyses given.

Oral toxicity study on rats

This study used only 5 male and female rats per group, which is an completely inadequate number to determine the true toxicological effects of GM brinjal on these rats. To give just one example of how inadequate this is, the concentration of a key liver function enzyme in the blood, AST, gives a measure of the health of the liver. Male rats fed GM brinjal had a concentration of AST that was 48% and 63% higher than feeding rats non-GM brinjal. Yet, this clinically significant finding was not found to be statistically significant. Calculations indicate that adding just a single extra rat to each group to bring the number of rats to a still tiny 6 per group, would have made this difference statistically significant, which would in turn have indicated that feeding GM brinjal to male rats could cause liver damage.

It appears that only one dose per rat was given and then the rats were followed for only 14 days. Food consumption, and only some haematology and biochemistry measurements were taken. It is normal to take 18-20 clinical biochemical measurements on blood from animals and humans to determine health. Yet only eight standard biochemical results are shown in the tables associated with this study. Only overwhelmingly adverse effects could be picked up this way using this number of animals for this time period and the study is simply inadequate to predict the effect of feeding this GM crop to 1.15 billion Indians for generations. Moreover, the company rarely reports the nature of the tests undertaken, the means, standard deviations, statistical tests undertaken or the p-values of the statistical analyses.

Animal feeding studies

Several animal feeding studies are presented in an effort to show that Bt brinjal is safe to eat. They include studies on fish, chickens, goats, rabbits, cows and rats. Most of these species are most unusual to use for human health studies, and many of the measurements taken on these animals are also unusual measures of human health. For example, chickens and fish are not even mammals. Chickens fly, lay eggs and do not suckle their offspring, swallow stones and grit to help grind their food, do not have human-like lungs or digestive systems and have kidneys that do not even produce urine. As chickens are clearly very different from humans, they therefore cannot be used as a model for human health. Using fish is worse. Besides the obvious differences in physiology involving things such as scales, lungs (humans cannot breathe underwater), and kidneys (fish kidneys do not produce urine), they are not even

warm-blooded animals. Many of these studies use death as an end-point. Death is not a measure of health. Most people know people who are alive but not healthy because they have serious illnesses such as cancer, diabetes, heart disease, liver disease or infectious diseases. Realistically, these studies are more useful to reassure primary producers that if they feed their fish, chickens, goats, rabbits and cows the GM brinjal, their animals will grow large enough and survive for long enough for the animal to get a good price at market. Further evidence for this is given by the emphasis on measures such as death rate, weight gain, growth rates, feed conversion ratios, milk production and carcass yield in these studies.

Furthermore, there was no full description of the diets fed to the animals in any of these studies. There was no list of the macro-nutrients used such as carbohydrate, fat, protein (and the components of these, such as the nature of the amount and type of saturated and unsaturated fats and which plants or animals they came from). Nor were the micro-nutrients given, such as the levels of the various vitamins and minerals in the diet. Nor was there a full description as to the source of the components of the diet such as which grains were used and in what proportions. So there is no understanding as to the nutritional adequacy of the diets. Furthermore, there is no understanding as to whether the diets were heat-treated before they were fed and how much heat may have been used. Heat can destroy proteins and anti-nutrients which might otherwise affect health. In addition, it does not appear that the various diets were analysed for other GM ingredients. Corn and particularly soy are often ingredients in laboratory diets and soy is certainly present in the fish and cow diets used by Mahyco. Much of the soy produced in the world comes from the US and South America and much of this is GM. The presence of GM products such as GM soy is therefore a confounder in these studies and needs to be measured. It is possible that any effects due to eating GM brinjal could be swamped by the effects of animals eating GM soy.

The number of animals used in each of these experiments is also too small to be able to find statistical significance for anything but overwhelming clinical findings. Often there are only five or six animals per group. To use a simple example of how inadequate this is, if the death rate is compared between two groups and six animals are used in each of those groups, two thirds (67%) of animals have to die in one group and nil in the other before a statistical difference can be found. If only five animals are used per group, the situation becomes even worse. Now 80% of animals have to die in one group and nil in the other before statistical significance can be found. There is also no statement as to whether the animals used were inbred or outbred animals. The use of outbred animals generally requires more animals in each dietary group for most measures to obtain statistical significance compared to using inbred animals. It is unlikely that Mahyco could source inbred laboratory fish, chickens, goats or cows.

While Mahyco's studies often report that measurements such as clinical chemistry were taken on blood, the results are rarely given. And even when they are given, it is unlikely that statistical significance could be found, given the low number of animals in each group. Organs may be weighed and perhaps expressed as a percentage of the body weight, but a diseased organ can weigh much the same as a healthy one. Histology, where the organ is sectioned, stained and looked at under a microscope is the appropriate method of determining if an organ is healthy. Yet this seems to have been rarely done.

The only real health study that could be used by Mahyco to support its application for safety

is a single rat study, which is why the company submitted the raw data associated with this study to the Indian government. In this instance, 10 rats per gender were used, the highest number of animals per group in any experiment. Again, studying this number of rats for only a few weeks is clearly woefully inadequate to determine the long-term health effects of 1.15 billion Indians eating GM brinjal for generations. An example of the inadequacy of the study's statistical power to find anything is shown by considering two matters. First, calculations indicate that if the number of female rats per group was increased to just 13, the 67% higher white blood cell count in the GM brinjal-fed group compared to a non-GM-fed group could reach statistical significance. Second, if the number of rats were increased to just 16 per group, GM brinjal could be found to cause a significant difference (increase) in AST in blood. This result supports the previous finding from the rat toxicological study where if 6 rats per group had been used, male rats could have been found to have a significantly higher level of this liver enzyme. Put together, the results of the two rat experiments indicate that if more animals had been used, male rats may have been shown to have evidence of liver damage from eating GM brinjal.

The raw data of this study indicate that the rats were highly variable at the beginning of the study. The body weights of some groups varied by as much as 31% **within** a group at the start of the study. This is an unusually high amount of within-group variability for body weight, and with a sample size of only 10 per group, could have masked any between-group effects. Essentially, statistics is about finding a signal amongst the noise. If there is too much noise, the signal cannot be found even if it is strongly present. Having this much variability within each group adds noise, making it very hard to find any signal.

The blood biochemistry and haematology data are also quite limited. For example, it is normal to take 18-20 biochemical measurement in blood to determine the health of an animal. This study takes only seven.

Moreover, while Mahyco presented a lot of raw data for some studies, with its interpretation of that data, it left-out most of the data that would be required in a peer-reviewed scientific journal for most of its studies and when data was actually given, Mahyco often omitted a key part of the analysis, such as the actual statistical results, eg p-values. That is, Mahyco omitted much of the results of the research from the report. It is also clear that the researchers were not blinded as to which group was fed GM and which was fed non-GM diets, which could bias the results. Moreover, the environmental conditions under which the animals were kept appear to be unusually variable and the GM status of the feed was determined using an inaccurate protein method instead of a far more accurate DNA method.

It appears that none of these studies has been published in a peer-reviewed scientific journal. This may be because the studies were not of a sufficient standard to be published.

Summary

While it appears the Mahyco has conducted a number of studies to show that Bt brinjal is safe to eat, in fact none of the studies are of any real use, for the following main main reasons:

1. The type of studies undertaken are insufficient to be able to determine if GM brinjal is safe to eat. For example, there have been no reproductive studies and the studies that have been done often use animals and/or measurements that are inappropriate or insufficient measures of human health.
2. Of those studies undertaken, the methodology and results are often insufficiently reported to be able to determine what the studies were actually measuring or how various variables were measured. Included in this, the statistical results have not been reported to a suitable standard. For example, means, standard deviations, and p-values, which would be required for any peer-reviewed scientific journal, are usually omitted.
3. The sample sizes are insufficient to be able to find statistical difference for many measurements even if real clinical differences are occurring between groups. Indeed, much of the research presented by Mahyco could be regarded as being burdened with Type II error. This type of statistical error occurs when sample sizes are so low that the study cannot realistically be expected to find a difference between groups of animals even if clinical differences are occurring.

Consequently, the studies presented by Mahyco cannot be used to show that GM brinjal is safe to eat, particularly when population health issues are taken into account. That is, if this GM brinjal comes into the Indian food supply, then every Indian will be eating it, resulting in 1.15 billion Indians exposed to the GM brinjal. Some of those exposed will be children or the elderly. Some of those exposed will already be ill with cancer, autoimmune problems, heart disease, diabetes, or infectious diseases. Because of the number of people exposed, if GM brinjal is later found to cause illness, it could cause significant economic and social problems for India. For example, if only 1 in 1,000 of exposed people later gets ill, or has an underlying illness made worse, then over a thousand million Indians would be ill and requiring treatment. This would result in a huge cost to the Indian government and community. It is therefore important to ensure that the safety assessment of GM brinal is sound and thoroughly covers all the major concerns of toxicology, allergy, and reproductive health. The studies presented by Mahyco are simply inadequate to determine these matters.

References

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