Annexure to *Commentary* entitled

Insufficient regulatory supervision prior to release of genetically modified crops for commercial cultivation in India

Pushpa Mittra Bhargava
Anveshna, 1-7-24, S S Nagar, Street No. 8, Habsiguda, Hyderabad 500 007, India
(Email, bhargava.pm@gmail.com)

***J. Biosci.* 34(2), June 2009, 167–168 © Indian Academy of Sciences**

Tests that must be done on GM plants intended to be released in the environment

(1) Using controlled field trials and studying the impact on ecology (for example, on populations of bees and other useful insects).
(2) Monitoring gene flow from transgene-carrying organisms to other organisms.
(3) Guarding against dispersal into areas where harm could be done (as happened with water hyacinth and parthenium)
(4) Studying the stability of the transgene product in the whole organism and/or parts thereof under various conditions of storage or handling (e.g. cooking in case of an edible genetically-modified organism [GMO]).
(5) Monitoring the effect on soil microflora.
(6) Testing for allergenicity.
(7) Carrying out acute toxicity studies with native (not "surrogate") protein, genetically-modified (GM) seeds and other GM plant material that is normally ingested by animals, including cattle. These studies should be done both on experimental lab animals and on farm animals such as goat, sheep and cows.
(8) Carrying out chronic toxicity studies (including carcinogenicity) as above.
(9) Studying the effect on microflora harboured by cattle.
(10) Looking for effects on soil micronutrients in regions where the GMO is likely to be released or find its way.
(11) Comparing the growth characteristics of the GMO and the parent organism.
(12) Looking for the emergence of new dangers, for example of 'super weeds', following prolonged use of herbicide-resistant GM crops.
(13) If the GMO is a plant, measuring its biomass productivity in comparison to the parent.
(14) Comparing the inputs required for optimal growth of the GMO relative to the parent organism and comparison of the relative cost: benefit ratio (this should include financial inputs and social costs).
(15) Monitoring the effect on useful insects.
(16) Monitoring the development of resistance to the trait for which the plant is genetically modified.
(17) Studying the increase in requirements for refuge crops.
Refuge crops are usually non-GM crops planted along with GM crops, for example, to provide an environment in which resistant pests might be out-competed by the normal (sensitive) pests.
(18) Studying a possible increase in the susceptibility of GM crop to pests and infectious agents other than those that may be expected to be killed or inactivated by the effects of the transgene.
(19) Studying the effect on the populations of non-susceptible pests following at least five successive plantations in the case of GM plants carrying the *Bacillus thuringiensis* (Bt) toxin gene.
In many cases involving successive plantations of Bt crops, the density of pests that were not originally susceptible to the Bt toxin increases. The number five is used as a guideline on the basis

of published data on the progressive increase in the population of pests that were initially known to be resistant to the Bt toxin.

(20) Carrying out a statistically validated programme involving the karyotyping and gross chromosomal analysis of genetically modified food plants and their consumers.
Chromosome alterations, e.g. translocations, can lead to serious health problems.

(21) Monitoring reproductive interference.
This refers to a change in the reproductive capability of an organism consuming the GMO or a product derived from the GMO.

(22) In the case of GM food material, possible interaction with commonly used drugs.
Drug-drug interaction is today accepted as an important issue in medical practice. A new or altered protein could have a drug-like effect.

(23) DNA fingerprinting and proteomics analysis, and characterisation, both structurally and functionally, of new and altered proteins.
It is established that genetic engineering leads to a higher rate of mutation than conventional breeding. DNA fingerprinting may (of course, not always) pick up some of the mutations. Functions in a cell largely depend on its protein profile. The only way to pick up changes in cellular protein profiles is through a proteome analysis which would identify new, altered or deleted proteins. Sequence comparison with known proteins in the protein data base (coupled with the knowledge we have of structure-relationships in proteins) can give some idea of the possible function of a new or altered protein – for example allergenicity, examples for which exist in the literature.

(24) Sequencing of transgene-flanking regions and the transgene; identification of the site(s) of integration of the transgene in the GMO.
The region that is sequenced should be large enough to identify the nature of the site of integration. For example, if insertion of the transgene takes place at certain invariant sites in the telomeric region, there could be serious problems.

(25) Identifying changes in the glycosylation pattern of proteins, which are known to occur in GMOs (and can affect the function of the protein).

(26) A study of the transcriptome.
Changes in transcription pattern can lead to changes in proteins and thus changes in their functioning. This is related to items 2 and 3 mentioned above and is intended to provide similar information pertinent to safety assessment.

(27) Monitoring changes in the relative concentration of major and important intracellular metabolites and precursors.
Normal ranges are available in many cases or can be easily obtained. For example, the free amino acid profile of a cell is generally reflected in the gross amino acid composition of the total protein in the cell. A major change in the concentration of just one amino acid can lead to translational errors and changes in the protein profile (apart from influencing pathways via feedback etc.). The metabolites and precursors chosen will depend on the particular case.

(28) Monitoring changes in surface properties that may affect normal interactions between species, and with the environment.
This can be studied through techniques such as scanning electron microscopy, atomic force microscopy and fluorescence-activated cell sorting (FACS). The cell types chosen would depend on the GMO and its projected use.

(29) Development in the country (if not already available) of a technique to determine with accuracy, even a 0.01 % contamination with GMO or its product.
0.01% is the level of contamination with a GMO that can be reliably detected with today's technology. It is also the limit of contamination of non-GMOs, by GMOs, permitted by the Government of India.