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SAFETY EVALUATION OF GENETICALLY MODIFIED FOOD: INDIAN REGULATORY OVERVIEW

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ABSTRACT

In India all genetically modified (GM) foods are evaluated under the Environment (Protection) Act 1986 and the Rules 1989. The Rules require that all GM organisms and products thereof are introduced only after they are found to be environmentally safe as well as safe for use as food for human and animals. GM food can be non-propagating substances of GM origin or GM organisms themselves such as GM edible yeast or lactobacillus or a host of agricultural produces such GM corn, potato, tomato, soybean, wheat, rice, mustard/rape seed etc. Bt cotton was the first and the only GM crop that has been approved for release into the open environment in India. The clearance was accorded under Rule 7-10 of the 1989 "Rules for Manufacture, Use, Import, Export and storage of hazardous microorganisms/Genetically Engineered Organisms or Cells" notified under the Environment (Protection) Act, 1986. All clearances are conditional and are for a limited period; renewal is required after the period is over. The law is based on precautionary principle. This paper describes what data are to be generated for transgenic plant-based food, what are the spectra of scientific debate on the issues, what major data have been generated globally, what issues are there before the Indian government and briefly, what was done to access the food safety of Bt-cotton in India. The status of development of GM food derived from animals has also been mentioned.

INTRODUCTION

Evaluation of safety of genetically modified (GM) food is an emerging area. Ensuring safety of such food to the public is the responsibility of the Government. The aim of safety testing of GM food is to introduce safe GM food into the market. The main objective of safety testing is to define the safety profile and to generate information in accordance with the profile to enable the entrepreneurs to obtain regulatory clearance for such food before they are commercially introduced. Three different kinds of GM food are under development and use. They are GM crops, livestock and poultry, fish and microorganisms**.**

Regulatory Guidelines:

In India, use of all GM food is controlled (11, 28) under the Environment (Protection) Act 1986 and the Rules 1989. The Rules require that all GM organisms and products thereof are introduced only after they are found to be environmentally safe as well as safe for use as food in human and animals. The Rules cover GM food, as these are products of GM organisms. GM food can also be GM organisms such as GM edible yeast or lactobacillus or a host of agricultural produces such GM corn, potato, tomato, soybean, wheat, rice, mustard/rape seed etc. They also include GM animals of diverse kinds like, for example, cat fish or carps containing growth hormone genes. The requirement under the Rules 1989 is to generate information on the safety of GM food in a prescribed format and the information is to be submitted to the Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment & Forests, which is the authorized agency for granting approval for commercial release. Generation of scientific data for GM food of diverse kinds is

authorized within the country in the research mode by the Review Committee on Genetic Manipulation (RCGM) of the Department of Biotechnology (DBT) Government of India, constituted under the Rules. In country data is generated on the basis of protocols approved by the RCGM. The data are placed before the GEAC, based on which it takes a decision on whether to approve or to reject a GM food for commercial use, under the Environment (Protection) Act (EPA). An approval implies that the approved GM food does not have any significant environmental impact, and the food is safe for consumption by human and animals.

Food is what one feeds on. Food is a substance, which on being digested nourishes the body, sustains it and promotes growth. In order to deal with these aspects of food, the Ministry of Health and Family Welfare have promulgated the, the Food Safety and Standards Act, 2006. However, the PFA Act did not deal with the GM food earlier. Rules have now been framed under PFA. These Rules would not only deal with the efficacy of the food but would also address their safety. They would also deal with issues related to identification of GM traits, either qualitatively or quantitatively or both.

Testing of food safety under the EPA is different from testing food safety under PFA. Besides safety, PFA would also require to address the issues related to identification, packaging and consumer's choice. Both the authorities need to examine and provide clearance, where after a GM food would qualify for use in Indian market. This paper describes what data are to be generated for transgenic plant-based food, what are the spectra of scientific debate on the issues and what was done to access the food safety of Bt-cotton in India.

Safety Testing of GM food under Indian EPA:

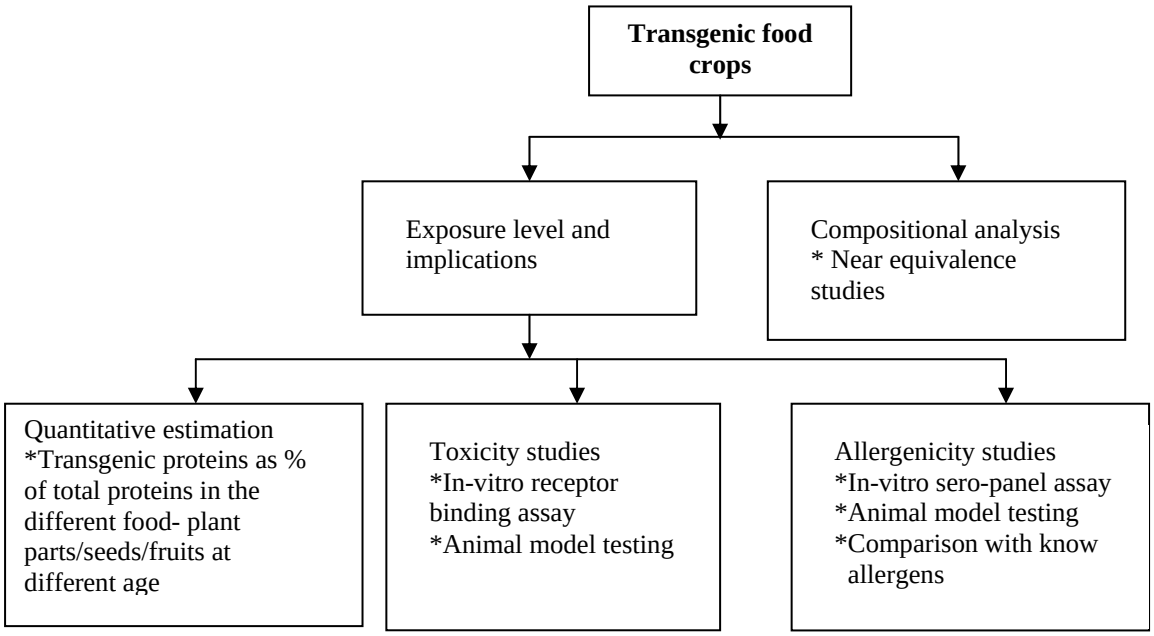
The generation of information emphasizes quantitative production of transgenic traits including transgenic proteins, and their effects on as-is-where-is basis on experimental animals in the context of determining the toxicity, allergenicity and anti-nutritional properties. It also emphasizes compositional analysis in order to complete near equivalence studies, also known as substantial equivalence studies. Such studies become more successful when the following conditions are satisfied:

a) The safety studies should be able to address the quantum and duration of exposure to human subjects for manifesting toxicity or allergenicity effects, or the studies should be able to state that within the imaginable level of exposure/intake there would be no observable effect on the human subjects. Data generated in small lab animals should include short, medium and significantly long-term exposures. Relevant animals should invariably be used to generate the basic data.
b) Wherever GM foods are implicated with adverse reactions, the science of adverse effects should be manifested and the target organs or systems showing the adverse reactions should be identifiable. The cells and tissues should be examined not only by histopathological methods but also by electron microscopic methods in order not to miss out the probable small differences, if any, being manifested during the regulated studies.
c) The feed should be so formulated with transgenic materials as well as with controls that both are isocaloric and are identical with respect to all measured components.
d) There should be unique identification criteria for identifying adverse reactions like manifestation of toxicity or allergenicity or changes in cells/ tissues at specific sites.
e) The adverse reactions should disappear preferably reversibly once the GM food is withdrawn.
f) There should be the possibility of extrapolation of high and low levels of exposure.

g) The data generated in animal species should be based on sound metabolic congruence in different relevant experimental animals.

In actual practice, however, conditions are never ideal and therefore, the above conditions are not satisfied. Yet data are generated on present knowledge based on sound scientific logic so that there is some possibility of drawing correlations between the observed findings in animals and the logical extrapolations of the results in human subjects.

Table 1: Information needs for assessing safety of transgenic food crops



Data are generated on a case-by-case basis on GM foods taking into consideration documentation on the following points:

i) Description of the GM food including source and sequence of transgenes, sequential block diagram of all the trans-nucleic acid stretches inserted, cloning strategy, characteristics of expression vectors, characteristics of promoters, level of expression of trans-genes, the transformation events and biochemistry of expressed gene products.

ii) Effect of the GM food on model microorganisms or mammalian cells if the food is implicated with toxicity.

iii) Descriptive toxicity and allergenicity studies, including study of local toxicity, systemic toxicity, reproductive and developmental toxicity, genotoxicity and mutagenicity, carcinogenicity and allergenicity or hypersensitivity testing. In some cases neuro or immuno-toxicity studies may be required.

iv) If the source material is implicated with known allergens, cautions must be exercised and sequence homology of the amino acids need to be determined or accessed from the available bioinformatics databases. The immuno-reactivity of the expressed proteins needs to be determined from the sera of allergenic individuals, if available. The kinetics of degradation of the proteins in artificial gastric juice as also in varying pH needs to be determined. Heat stability of the proteins is also assessed. The decision process is influenced from these data.

The Indian Guidelines (25, 26, 27) that have been developed for testing the safety of transgenic plants that enter into human food chain require generation of toxicity and allergenicity data generation in small adult laboratory animals. The Guidelines specify the number of animals and their types to be selected for conducting specific safety studies. The data are generated usually by classical animals feeding trials. Immuno-toxicological studies as well as gut toxicological studies are also carried out wherever required.

Schematically, the information needs (4,5,7,8) for evaluating different kinds of transgenic food crops can be represented as depicted schematically in Table 1.

The composition analysis for substantial equivalence studies for assessing near equivalence in composition requires the determination of various components such as proteins, carbohydrates, lipids, oils, fiber, moisture, minerals etc. The OECD and the WHO guidelines elaborate on the concepts of substantial equivalence as has been summarized in the box below.

Box: Concept of Substantial equivalence

Substantial equivalence professes the idea that if an rDNA product is considered substantially equivalent to an existing product then safety assessment of the rDNA product should be made in the context of the existing product. A novel food is considered to be essentially the same as that found in nature except for the novel trait. Such a concept is not new as the same principle was used in the past in assessing the safety of new hybrid varieties produced by non-biotechnological means.

The rationale of substantial equivalence does not require the testing for toxins that would not logically be present; this argument would avoid the folly of testing animals with whole foods that might demonstrate several drawbacks like nutritional deficiencies, at the highest dose levels, which symptoms may result from food displacement or other effects unrelated to the change rendered by rDNA manipulations.

Because the extents of equivalence to a previously consumed food is a continuum of "substantial" to unique and were not existing in nature, the degree of equivalence has been proposed on a three tier model as elaborated below:

- When substantial equivalence has been established for an organism or a food product, it is considered to be as safe as its conventional counterpart. No further safety evaluation is needed in such situations.
- When substantial equivalence has been established except from certain identified and specified differences, further safety assessment should focus on these differences; a sequential approach may be made, which may focus on the new gene product(s) and their structure(s), function, specificity and use history if any. Further *in vivo* and/or *in vitro* studies may be quite rational if a potential safety concern is indicated for the new gene product(s).
- Food products are not necessarily considered unsafe even if the substantial equivalence cannot be established. All such products will not require extensive safety testing. The testing program must be established on a case-by-case basis on the merits of each case. In such cases, further studies, including animal feed trials, may be required, especially in situations where the new food is intended to replace a significant part of the diet.

Wherever the products are implicated with toxic substances, the change in their concentrations between the GM food and their non-transgenic controls is measured. The exposure level determines the level of expression of transgenic proteins and toxicity as well as allerginicity studies under different connections.

Qualified and trained personnel using precise equipment carry out all testing. Adequate care is taken in the use of animals for tests. All tests are based on standard operating procedures following an approved protocol. The Regulatory Authorities approve protocols. There is proper documentation of all the events and observations during the conduct of the studies. The raw data are to be preserved and may be subjected to scrutiny by the authority wherever required. The standard operating practices are to document procedures for sampling, animal handling, lab work and testing procedures. The practices also elaborate procedures for calibrating and maintaining sophisticated equipment used for data generation. Data handling procedures and reporting methods are also documented in the operating practices. Wherever GM food is implicated with adverse reaction to the operating personnel, procedures are evolved for the safe handling of these during testing.

Procedures for interpretation of experimental results:

There are several tools that are used for the interpretation of the safety evaluation data. These include kinetics of the transgenic analytes (substances) studied in animal model and identification of factors responsible for toxicity or allergenic manifestation. Statistics plays an important role in interpreting the results. However, much depends upon the knowledge of the evaluators who need to apply their judgement based on their experience. As GM food is a new area under development globally, often there is not enough exposure of people having adequate knowledge of handling GM food. Experts are therefore drawn from various Institutes dealing with nutrition of non-GM food who have exposure of studying adverse reactions including toxicity or allergenicity emanating from such food. Experts are also drawn from Institutes specializing in immunological studies. Decisions are often based on collective wisdom of experts and these take into consideration the advantages of the GM food, value of assessing risk verses benefit, judgement on statistical significance of findings verses biological significance and risks involved in marketing without a complete knowledge of the adverse reactions. It is to be remembered that all regulations are time bound and, therefore, a decision is to be taken on the basis of current scientific knowledge where the science of the safety of GM food is still evolving. Decisions are consequently based on the collective wisdom of knowledgeable decision makers. These decisions also take into consideration the societal needs and the economic impacts from the use of such GM food.

Health consequences of GM Food and Feed: some significant data generated globally:

By studies on laboratory animals, data have been generated at different laboratories all over the world to access the possible health consequences. These data also need to be studied to have insights in to what has been happening around. These data serve as reference points for conducting a fair and detailed further studies in Indian labs, where required.

Work has been carried out to a considerable extent of GM from GM crops. GM tomato expressing Bt Cry1Ab insect-toxic protein was used to prepare semi-synthetic animal feed and this was mixed with 10% tomato that were either GM or were the normal unmodified substance. The mixture was lyophilized and fed to young male and female rats for 91 days. The diets were about 20 gms of fresh tomato per day. After the feeding experiments the animal were sacrificed along with controls. The organ weights including kidneys, liver, testes were examined both for animals fed with transgenic and non-transgenic feed. Observations were also taken on the initial weights, the terminal body weights and the survival percentage in both the groups. There were no difference between the organs and tissues of animals fed with transgenic and non-transgenic

feed or any difference in terminal body weights or survival percentage (17, 19). The Bt toxins expressed were about 7.5 ng/mg of protein in the fresh GM tomato.

Rodent diet was prepared (9) using Monsanto's commercial RR soybean lines. Two lines designated as 40-3-2 and 61-67-1 were used. A commercially available rodent diet was used as the base. Reformulated diets were prepared to contain 25% of soybean, either from the GM lines or from the non-modified material. All the feed contained 25% protein. There was no difference in the terminal body weights as well as in the relative weights of vital organs including kidneys, liver and testes. There was also no major difference in pathologic findings. The feeds were also used on catfish. The high protein diet containing transgenic proteins showed satisfactory growth of rats, besides the catfish.

Two GM potatoes developed at Scottish Research Institute, each expressing the lectin GNA from snowdrop i.e. *Galanthus nivalis* were used to find their effects on rats (23). Male rats, 19 days old were fed a diet for 10 and 110 days. Four feeding groups of animals were devised. Feed contained potatoes up to 65%, 70% or 76% on a dry weight basis. Potatoes were either one of the two GM lines or the unmodified line, spiked with free GNA protein. Three control feed were used without potatoes and another three control feed were used which were standard rat feed. The protein content in the different feed was adjusted to 6%, 8-9% and 15%. After the experiments, the organs were studied. Differences in the weights of organs in different feed were reported in the study using a detailed statistical analysis. Subsequently, measurement of the mucosa of the stomach lining as well as the crypt lengths of the intestine at various points were taken and reported (22). These data showed deleterious effects from transgenic potatoes. The histopathology study conducted on a follow up work (20) showed hyper plastic growth of small intestine of rats fed with GM potatoes. The jejunum of the rats were not enlarged however, and the results were consistent with the author's earlier studies (21). In another study by this group on GM peas, expressing alpha amylase inhibitors, diets with 15% proteins were prepared. These diets contained either 30% or 65% peas. The peas were either from GM ones or from the non-transgenic parental pea lines. Diets were also made that did not contain any peas at all. These different kinds of feed were fed to male rats (10 days old) for ten days. All the animals were sacrificed and their small intestinal lumens as well as the caecum tissues were analyzed. Final body weights as well as the organs were also analyzed. The organs and tissues of rats fed with GM peas and the ones fed with the non-transgenic parental peas did not show any significant difference except for results of feed of 30% dietary inclusions; at this feed intake, the caeca and the pancreas were significantly different. The nutritional performances were also quite different (22).

A Japanese group conducted a feeding study, using two GM potatoes, each expressing a glycinin. One of the modified potatoes expressed the natural glycinin and the other expressed a modified protein with four methionyl residues. Four groups of male rats (4 weeks of age) were used in the experimental design. A commercial diet was fed to all the rats. Two of the groups of rats each, were fed with about 0.16 grams of mashed, GM potatoes per animal daily, where the feed was introduced through a metallic gastric tube. One another group was also similarly fed with non-GM mashed potatoes. In other words, three groups received potatoes while one group had only the commercial feed. The details about the body weights, organ weights, histopathological findings and hematological data were generated and examined. No differences were observed among the four groups (10).

A diet containing 67% corn of Novartis' Event 176 GM-corn, expressing Bt Cry1Ab was prepared. Another diet with unmodified parental corn line was also prepared. These two feed were fed to the male and female broiler chickens (1 day old) for 38 days. The protein contents in the diets were 230 g/kg. There was

no difference in percentage of survivals of chickens nor in their body weights between the ones fed with transgenic feed or with the non-transgenic material (1).

In a study using GM potatoes expressing Bt Cry1Ab δ-endotoxin (produced in an Egyptian institute), one month old mice in three groups were fed for two weeks. One group was fed with non-GM potatoes, one with GM ones and the other with potatoes treated with free δ-endotoxin. In all the cases after sacrifice, the ileums of the animals were studied both by light and electron microscope. It was observed that there was change in the structural configuration of the cells of ileum of animals fed with GM potatoes, as also with the tissues of the ones fed with potatoes treated with free δ-endotoxin (3).

In another study, using Monsanto's glyphosate tolerant soybeans i.e. Round Up Ready soybeans, an investigation was carried out using female B10A mice (7 weeks of age) and female Brown Norwegian rats (7 weeks). Three classes of feeds were devised, one containing 30% GM soybeans and 7% casein (total 17-18% protein feed); one with unmodified soybeans and another one as the standard, commercial rodent feed (CRF-1). The animals were fed for 15 weeks. After sacrifice, the organs and tissues were analyzed. No differences were found between animal parts fed with GM soybeans and those with the non-modified ones (29). The level of production of soybean specific IgE could not be detected in the sera of either group of animals. The increase in the soy specific IgG was the same in both the groups.

The review of the existing literature shows that in some of the studies, the adverse reactions from the feeding of the GM substances have been observed while in majority of the studies, the adverse reactions were not visible. First of all, it is to be borne in mind that GM plant materials express transgenic proteins in very minute quantities. For example, the GM tomato expressing BtCry1Ab toxin had shown only about 7.5 ng/mg of protein in the fresh GM tomato (17). The GM Bt-Cotton of Monsanto had also expressed small quantities of Bt Cry1Ac proteins in different plant parts in Indian experiments (6).

The above studies are not conclusive about the safety of transgenic plants. Some of the above experiments were rudimentary and more studies were considered necessary for drawing critical evidence about the safety of the transgenic analytes. Intestinal tissues from different parts were required to be examined closely to assess if local damage had taken place. In many experiments, the exposure time appeared to be too short. In many investigations, where damages were found from exposure from transgenic materials, more follow-up studies seem to be necessary. However, these studies could be used as foundations for building Indian strategies for conducting national food safety evaluation on transgenic substances.

Safety assessment of Bt-Cotton Seeds in India:

Government of India had authorized the commercial cultivation of Bt- Cotton in March 2002 after elaborate assessment of environmental safety as well as food safety of Bt- Cotton. For the evaluation of food safety, Bt -Cotton seeds were evaluated.

Bt cotton, a transgenic plant, produces an insect controlling protein, which is toxic to lepidopteran pests like bollworm and is named as Cry1Ac protein. The gene had been derived from the naturally occurring bacterium, *Bacillus thuringiensis subsp. kurstaki* (B.t.k.). The cotton hybrids containing Bt gene produces its own toxin protein for controlling the bollworm attacks. The Bt cotton plants significantly reduce the requirement of spraying them with chemical insecticides to protect the bolls from insect attack. This enables a major benefit to cotton growers and to the environment.

Bt cotton cultivars contain the following three transgenes inserted via genetic engineering techniques:

- The *Cry1Ac* gene, which encodes for an insecticidal protein, Cry1Ac, was derived from the common soil microbe *Bacillus thuringiensis* subsp. k*urstaki (B.t.k.*), as stated earlier. This gene is under the control of the promoter CaMv35S and is therefore, expressed in cotton plants.

- The *nptII* gene, which encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII). This gene was used to identify transformed cells that contained the Cry1Ac protein. It served no other purpose and has no pesticide properties. The *nptII* gene is derived from the prokaryotic transposon Tn5. The gene *nptII* is under the control of the promoter CaMv35S and is therefore, expressed in cotton plants.
- The *aad* gene which encodes the bacterial selectable marker enzyme 3"(9)-O- amino glycoside adenyltransferase (AAD) allowed for the selection of bacteria containing the plasmid PV-GHBK04. This gene enables selection of surviving cells in media containing spectinomycin or streptomycin. This gene *aad* is under the control of a bacterial promoter and is therefore, not expressed in cotton plants.

The NPTII and AAD proteins are used as selectable markers. They have no pesticides activity and are not known to be toxic to any species.

As Bt cotton is a transgenic crop, it requires environmental clearance under the Indian Rule 7-10 of the 1989 Order, entitled "Rules for Manufacture, Use, Import, Export and storage of hazardous microorganisms/Genetically Engineered Organisms or Cells", notified under the Environment (Protection) Act, 1986. Prior to the authorization of Bt cotton for cultivation in India, enough data and information were necessary to evaluate it from environmental safety. The environmental issues related to this novel substance was assessed from different aspects which, *inter-alia*, included molecular characterization of introduced genes, biochemical characterization of the expressed proteins, estimation of the level of the expressed Bt proteins in cotton, Bt proteins in cotton plant parts, safety of the Bt cotton plants to non-target organisms, environmental fate of the Bt protein like residues in soil if any and their effects on soil organisms, compositional analysis of cotton seeds and refined cotton oil as well as food and feed safety evaluation of Bt cotton compared to non-Bt cotton, using the Bt and non-Bt cotton seeds as substances for evaluation. Agronomic evaluation was also carried out for the Bt cotton to ascertain what economic advantages these cultivars provide to the farmers over the existing ones.

Studies to evaluate the Food Safety of Bt cotton

For evaluating food safety, the studies conducted include: compositional analysis, allergenicity studies, toxicological study, presence of Bt gene and protein in Bt cotton seed oil and feeding studies on adult lab animals, goats, fish, chicken, cows and buffaloes. Salient features of these studies are as follows:

Compositional analysis

The studies revealed that there was no change in the composition of Bt and non-Bt seeds, with respect to proteins, carbohydrates, oil, calories and ash content.

Allergenicity studies

Allergenicity studies were conducted in Brown Norway rates. No significant differences in feed consumption, animal weight gain and general animal health were found between animals fed with Bt cotton seed and no cotton seed. At the end of the feeding period, the relative allergenicity of traditional cotton hybrids and Bt cotton were compared to Bt and non-Bt protein extract in active cutaneous anaphylaxis assays. Results of the study concluded that there is no significant change in endogenous allergens of Bt cotton seed compared to non-Bt cotton seed.

Toxicological study

A goat feeding study was conducted for understanding the toxicological effects of Bt cotton seed. The animals were sacrificed and the vital organs were assessed for gross pathology and histopathology. No significant differences were found between animals fed with Bt and non-Bt cotton seed. Studies on goats were preceded by detailed analysis of the available data on adult lab animals. The data were generated utilizing Bt proteins produced in *E. coli.* Feeding data generated on the basis of *E. coli* proteins were considered non-representative, as they were not glycosylated, while the ones expressed in plants were considered to be considerably glycosylated, thereby rendering their biochemical properties to be different. Indian views were that the proteins as available in plants be used in feeding studies for obtaining more conclusive results.

Presence of Cry 1Ac gene and protein in Bt cottonseed oil

Studies had indicated that *Cry 1Ac* gene and protein were not found in refined oil obtained from Bt cotton seeds.

Feeding studies on fish, chicken, cows and buffaloes

Feeding experiments conducted with Bt cotton seed meal on fish, chicken, cows and buffaloes indicated that Bt cotton seed meal is nutritionally equivalent, wholesome and safe as the non-Bt cotton seed meal. The feeding experiments on poultry birds, fish, cows and buffaloes were conducted at National Dairy Research Institute (NDRI), Karnal on lactating cows; Department of Animal Nutrition, College of Veterinary Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar on lactating buffaloes; Central Avian Research Institute (CARI), Izzatnagar on poultry; and Central Institute of Fisheries Education (CIFE), Mumbai on fish.

From the above studies of Food Safety conducted on Bt-Cotton seed meal as well as refined Bt-Cotton seed oil it was concluded that Bt-Cotton seed meal did not have any significant effect of either toxicity or allerginicity different from non Bt-Cotton seed meal. The refined Bt-Cotton seed oil did not contain either the transgenic protein or the transgenic nucleotide sequences. It was therefore concluded that Bt-Cotton seed meal was safe as feed and refined Bt-Cotton seed oil was also safe and not different from non Bt-Cotton seed oil. On the basis of these evidences, the Government of India had authorized the use of Bt-Cotton freely for cultivation and use in Indian agriculture under the Indian Environment (Protection) Act 1986 and Rules 1989. As the long-term use of Bt-Cotton is not yet known, nor can it be predicted, the authorization of the Government has been conditional and was for a period of three years from April 2002 – March 2005. During this period, the Government was to monitor the crops and their effects during continued use. The Government accorded extension for further use of Bt cotton subsequently. This implies that the Bt cotton was doing fine in the environment and no human food safety concerns have surfaced yet. The REPORT of the study needs to be placed before the public for instilling more confidence about the continuous use of GM substances by the society.

Food derived from GM animals:

GM food under development is foods derived from GM livestock and poultry. These life forms were developed by introducing growth enhancing novel genes into pigs; there were morphological and physiological changes found in such pigs and therefore, they were not introduced in to the market (14).

Several kinds of genetic modifications have been proposed to develop engineered cows that produce either new proteins in milk or produce more endogenous proteins. Researchers from New Zealand have

developed GM cows that produce milk with increased levels of casein protein. In other strategies GM cows are being developed with the intention of producing milk containing reduced lactose content. Such animals have not yet been introduced for use in commercial animal husbandry practices (18).

There are other strategies that aim at developing animals with improved disease resistance and increased birth rates in sheep; increased egg production in poultry by creating two active ovaries; altering sex ratio in poultry; and creating improved feed conversion in transgenic pigs that excrete less phosphorous. These works have not yet culminated into release of GM animals for commercial use (14).

GM fish has been produced in contained environment; such fishes have high feed conversion ratio. Multiple copies of growth hormone genes have been incorporated in such GM fish. Such fishes are being consumed but have not yet been officially released into the open environment in order to prevent the transfer of transgenic sperms / eggs into the natural environment. In most cases, such GM fishes have lesser life than their natural counterparts (2, 13, 15, 16, 24, 30).

Food derived from GM animals should be assessed on a case- by- case basis and should include compositional analysis and phenotypic analysis. Model protocols need to be developed. Assessment should also include assessment of risks of potential recombination of viral vectors (which may have been used for the transformations) with the wild viruses found in nature.

Issues relating to the use of GM substances:

The issues relating to the use of GM substances are not simple. Although several short-term experiments have been conducted to instill confidence in such food, long-term studied is required for the use of many of them. Such instances as natural genetic transformation have been found to occur in different environments, where GM food was consumed. Ingested DNA from food is not completely degraded by digestion and small fragments of DNA from GM foods can be found in different parts of the gastrointestinal tract Horizontal gene transfer (HGT) as the consequences may be significant in some human-health conditions; therefore, the potential for HGT needs to be part of the risk assessment of GM food (14).

Non-viral gene delivery is a complex issue as there are barriers of transport of DNA pieces into the nucleus. There are certain peptides that are called as the nuclear localization signal peptides (NLSPs), which would bind with the target DNA pieces to carry the train of peptide bound DNAs beyond the border of nuclear membrane. Having entered into the nuclear space, the DNA pieces acquire the opportunity of either interacting with the RNA polymerase of the host cell on a stand – alone basis or get integrated into the genome when the host cell is in the dividing phase. Non-integrated DNA pieces can produce translating mature RNAs that can be exported in to the cell cytoplasm for translation. Integrated DNA pieces can also express themselves under certain circumstances; the integrated DNA (wherever this happens) creates mutations in to the chromosome, which if not repaired or eliminated may manifest deleterious effects on the host organisms.

Certain viruses or their pieces have coding DNA sequences containing strong promoters as well as enhancers in them that promote their transformation into c DNA at ease in the host cytoplasm; the resulting c DNA gets bound with the NLSPs and get transported across the nuclear membrane again at ease, with the assistance of signal peptides of their own or those hijacked from the host cells. Such c DNA pieces when bound intact with open reading frame and an enabling terminator sequence (stop codons) can transport the whole train of the coding sequences intact along with them to the nucleus; the border barriers of customs get easily deceived when such sequences sneak into the nucleus in the presence of the natural guards that are entrusted to prevent illegal entry, as the signal peptides play the role of touts.

Interestingly viral vector DNA pieces from SV-40 have this ability to transport replicating DNA along with them without the assistance of the cellular nuclear localization signal peptides. Other DNA sequences of viral origin, which are rendered replication deficient such as those from CaMv35s, HIV, HBV, Adeno, Vaccinia and many others get into the nucleus of varied karyo types as soon as they have entered into the cell cytoplasm. They are therefore, to be considered as potentially hazardous! Different groups all over the world to produce transgenic substances have utilized several promoter sequences of viral origin. The CaMv35s promoter has been extensively used in plant transformation studies. The issues emanating from their use should therefore, be considered as parts of food biosafety.

Future Issues and the Indian out look:

Since the Indian Environment (Protection) Act 1986 and Rules 1989 do not require handling the issues related to the choice of food to the consumers, the Government did not examine the factors such as the rights of consumers to choose between transgenic and non-transgenic food, when Bt cotton technology was approved in 2002. These issues are now being addressed through the Food Safety and Standards Act, 2006 enacted by the Central Ministry of Health and Family Welfare, New Delhi.

It is anticipated that as the number of transgenic food products increases in the Indian Market, there would be the need to address these questions very transparently and legally. As these issues are very complex, it is anticipated that there would be interesting public debate. The question of mandatory or voluntary labeling of transgenic food would have to be settled with adequate clarity for the society in order to enable the latter to make a choice for the consumption or otherwise, of GM food.

Since GM food may have factors related to their effect on long term use in humans and animals, the Indian EPA may also have to address the questions of labeling in the coming years. In parallel, the Food Safety and Standards Act, 2006 may also handle such issues legally. As and when such issues are given a legal status the question of packaging development, accompanying documentation for transport and handling in- transit as well as during use individually or in combination would also come into play. The future years would be interesting to watch as to how such situations are handled in the Indian legal framework. It is obvious that labeling of GM food imposed legally or carried out voluntarily, would not be a simple exercise. It is stated in this context that, if the food safety issues are resolved beyond doubt before GM foods are approved for use, it may not require labeling to indicate hazards, as safety assessment would have already addressed such issues. In such cases, if labeling is avoidable then only their introduction would be easier. Every country dealing with issues of labeling has enormous administrative problem and often labeling requirement does not become fool proof. This is the experience world over including in China. The alternative is to apply voluntary labeling provisions in order to enable a consumer to make a choice in situations where the safety questions have already been resolved scientifically. The future years are therefore going to be pivotal in facilitating or preventing the introduction of GM Foods for consumers and the balance will swing towards facilitation if liberal labeling policies are adapted.

Generation of food safety information in the public institutions is felt very strongly in the country. Indian government has spent substantial sum in R&D for the development of GM crops in Indian national institutes. However, these expenditures have yielded very little results as yet. Transgenic materials have been sourced mostly from foreign sources. Use of such materials is restricted for research purposes only. Material Transfer Agreements (MTA) are one-sided. MTA needs to be devised in a manner at the political and diplomatic level that enables the use of transgenic materials by the Indian public funded institutions freely for

public causes. Many negotiating factors exist in favor of this for India. While encouraging public-private collaborations for developing new materials, the benefits of intellectual property protection in favor of the public institutions need to be remembered.

Food safety data should be generated using public institution-derived materials at public institutions. Private materials should be evaluated in public funded institutions without dependence on private collaborations. Studies carried out without collaborations with private institutions shall have more credibility before the opinion makers. All food safety data generated institutionally or in collaboration with public-private collaborations need be published in peer-reviewed scientific journals. Infrastructures need to be substantially strengthened for this.

A set of guidelines need to be developed for conducting newer experiments on food safety of transgenic crops and materials produced from transgenic substances, which would improve/ modify the already existing guidelines (26).

Training of the evaluators working at the authoritative level needs concerted attention. While the existing system is extremely comprehensive, lack of adequate number of competent people has made the system somewhat sluggish. This needs to be attended to instead of creating alternative infrastructures.

Alliance at the Indian government level with the international bodies such as the Food and Agriculture Organization (FAO), World Health Organization (WHO), Organization for the Economic Co-operation and Development (OECD), the International Life Sciences Institute (ILSI) etc, which are also involved in the development of food safety guidelines for GM food, by global consultations and agreements may often have much to say and guide the nations in the matter. Countries including India that are resource poor and may not be able to generate all the infrastructures to handle all the relevant issues on the safety assessment of GM foods may bank upon such institutions during the initial stages through different kinds of teaming up arrangements.

Concluding remarks:

The feed intake of experimental animals with reference to transgenic proteins would generally be insignificant and therefore, one could expect results of insignificant variance between the animals fed with transgenic materials versus those fed with non-transgenic substances, unless the experiments are carried out for a long period thereby implying effect of long-term exposure. Adverse impacts would also be manifested if the transgenic proteins were indeed, severely toxic/allergenic to mammals/ human. The duration of study of experimental animals needs to be standardized with reference to different transgenic substances. It is anticipated that the duration of exposure may play a significant role in the manifestation of adverse effects. Transgenic proteins produced in the same host organism / transgenic plants need to be used for generating safety data than using transgenic proteins, produced in microorganisms like *E. coli*. Post-translational modification of the proteins occurring in the cytoplasm of different eukaryotes may produce glycosylated products with different kinds of sugar residues. There are also expectations that there would be considerable variations in the degree of N-glycosylation and O-glycosylation in the dressing up of the final proteins in the cytoplasm. Variations in Sialic acid content could be another factor that may lead to differences in the properties of certain kinds of proteins. The preponderance of the extent of differently glycosylated proteins of the same aminoacid sequence is yet another factor that can make a variation in the safety assays. The posttranslational modifications could modulate the binding properties of proteins to the target cells. They can also influence their elimination/degradation kinetics. Intact trans-nucleotide sequences containing the

promoter, the gene and the terminator sequences getting into the body may express, if they get into cell-cytoplasm. Therefore, proteins and trans- nucleotide sequences that can cross the intestinal barrier through several segments of the ileum through endocytosis or diffusion or any other manner need to be examined carefully. Base line data for such phenomenon need to be developed first. Taken together, all these properties need to be looked at when experiments are designed, data collected and results interpreted. Therefore, while initial introduction of transgenic substances could be based on relatively simple experiments for assessing short- term safety, concomitantly long-term safety data need also be generated. The authorities must, therefore keep a watch on the performance of introduced transgenic substances, living or non-living. Commercial introduction has therefore to be conditional and time restricted, which would need renewal from the authorities after their expiry, for the applicants' remaining further in to the market.

The present day evaluation of GM food takes into consideration the existing scientific knowledge. The level of existing knowledge needs to be continuously improved. The current span of evaluation is often based on short-term study of risks. The animal model used for the study of toxicity and allergenicity is not always considered to be ideal for co-relating the results generated in rodents or higher animals that are extrapolated to human subjects. No safety studies are conducted in human before the GM food is introduced commercially. There is little guarantee that where the small number of human subjects that may manifest any adverse reactions from the use of GM food, such indications would be reflected adequately through the pre-marketing animal trails. Consequently, there is a strong need to keep a watch on post-marketing era of GM food to record adversities, if any, with a view to take corrective actions by the authorities. There are also possibilities that the consumer uses the GM food available in the market after modifying it by utilizing certain cultural practices that may cause adverse reactions. This also calls for keeping a watch on the introduced GM food. There is, however, great hope that already several GM foods have been introduced worldwide and use of none of them have caused any reported large-scale adverse reactions. GM food is the result of application of existing scientific knowledge of assessing a food or food products. Agricultural biotechnology and food processing that have collectively enabled agricultural practices more productive and food processing more economic hold the potential of making more food available to human kind at cheaper prices. It has been reported that the global market of GM crops has reached US $4.50-4.70 billion in 2003 and that the crops namely; soybean, maize, cotton, canola, papaya, tomato and potato have taken the maximum global share. The land use for transgenic crops in 2003 was 67.7 million ha and that the farmers from the developed countries grew 70% of the estimated global areas of GM crops (67.7 million ha.) and the rest 30% were planted by farmers in the developing countries. In 2006, the land use for GM crops increased to 102 million hectares worldwide (15) of which the contribution from the developing countries increased and was 40%. Consequently, GM technology shall be deployed more extensively in future in the developing countries where the need for food is more. In this context the safety of GM food need to be evaluated both on short-term as well as on a long-term basis. A precautionary approach with exhaustive scientific studies will assist the acceptance of such food by the society when they would otherwise be safe. Haste in release is expected to be retrograde in the societal acceptance process.

International organizations such as FAO/WHO/OECD are continuously conducting safety assessments with transgenic plants/ products derived there from, to provide assurance of safety of GM plants/ food products to the human kind. It would be wise to have continuous alliance with such organizations for keeping the national system current from their findings, recommendations and guidelines.

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