

CONFIDENTIAL

Final Report of the Technical Expert Committee (TEC)

Section I: Background Document and Scientific Rationale for the TEC⁵s Recommendations in its Interim Report

1-1. Preamble

The Order of the Hon'ble Supreme Court dated 10 May, 2012 in Writ Petition (Civil) No. 260 of 2005, Aruna Rodrigues and Ors vs. Union of India, directed the TEC as follows:

(2) The terms of reference of the said Committee shall be:

a. To review and recommend the nature of sequencing of risk assessment (environment and health safety) studies that need to be done for all GM crops before they are released into the environment.

b. To recommend the sequencing of these tests in order to specify the point at which environmental release through Open Field Trials can be permitted.

c. To advise on whether a proper evaluation of the genetically engineered crop/plants is scientifically tenable in the green house conditions^o and whether it is possible to replicate the conditions for testing under different agro ecological regions and seasons in greenhouse?

d. To advise on whether specific conditions imposed by the regulatory agencies for Open Field Trials are adequate. If not, recommend what additional measures/safeguards are required to prevent, potential risks to the environment.

e. Examine the feasibility of prescribing validated protocols and active testing for contamination at a level that would preclude any escaped material from causing an adverse effect on the environment.

f. To advise on whether institutions/laboratories in India have the state-of-art testing facilities and professional expertise to conduct various biosafety tests and recommend mechanism to strengthen the same. If no such institutions are available in India, recommend setting up an independent testing laboratory/institution.

g. The Expert Committee would be free to review reports or studies authored by national and international scientists if it was felt necessary. The petitioners opined that they would like to formally propose three Expert Reports from Prof. David Andow, Prof. Jack Heinemann and Dr. Doug Gurian Sherman to be a formal part of the Committee's deliberations. The MoEF may similarly nominate which experts they choose in this exercise.

The Interim Report submitted in October 2012 to the Honourable Supreme Court adhered to the following relevant portions of the Order;

(5) "In the event and for any reason whatsoever, the Committee is unable to submit its final report to the Court within the time stipulated in this order, we direct that the Committee should instead submit its interim report within the same period to the Court on the following issue:

Whether there should or should not be any ban, partial or otherwise, upon conducting of open field tests of the GMOs? In the event open field trials are permitted, what protocol should be followed and conditions, if any, that may be imposed by the Court for implementation of open field trials." (Emphasis by the TEC)

This section of the final report provides the scientific background for the recommendations made in the interim report of the TEC. It also responds to the Affidavit of the Ministry of Agriculture and to other queries as relevant made by various parties that were interviewed by the TEC during the period July 2012 to March 2013.

The other sections of the final report complete the consideration of matters under the TOR carried out by the TEC as instructed by the Hon'ble Court with recommendations.

1-2. The Ministry of Agriculture Affidavit to the SC and its Submission to the TEC

There are two submissions by the DAC of the Ministry of Agriculture: the first one to the Honourable Supreme Court in their Affidavit filed by the DAC, on 08/11/2012 which is a Reply to the Interim Report of the TEC and the second, a formal submission to the TEC ([DO.No.4-15/2011/SD-V](#), dated 13/12/2012). The submission to the TEC states the following on pages 3 and 4:

"The DAC has no direct role in policy matter related to research and development of GM crops, labeling for consumer awareness, assessing impact on biosafety and human health, livestock health etc. ... DAC keeps a watch on the research and development and supports biotechnological intervention for enhancing the production and productivity in agriculture".
and

"...as per the order of the Honourable Supreme Court dated 14 April 2011 para 6.2 (a) to (g) deals with the Terms of reference (TOR) for the Committee. The TOR in totality, raises technical issues related to aspect

of GM trials and DAC has no mandate in this regard. These technical issues fall within the Mandate of Ministry of Environment & Forest, Department of Biotechnology".

The submission of the DAC to the TEC appears to stand in opposition to its Affidavit in the Supreme Court. This matter is brought to the attention of the Honourable Supreme Court. Nevertheless the TEC responds briefly to the Affidavit later in the report.

Before taking up the TOR and discussion of the recommendations made in the interim Report, it is necessary for the TEC to place these issues in the context of the central position of agriculture in Indian society and to bring out some of the important features of Indian agriculture that need to be kept in mind when considering regulatory decision making. As will be elaborated in the report, the TEC is of the view that the regulatory process must include considerations based on the prevailing socioeconomic and need-based factors, taking into account the available alternatives, and to assess the impact the product / technology is likely to have in the Indian context and across the cross-section of Indian farmers.

Background and Context of the TEC's Recommendations in the Interim Report

India with its large biodiversity is a major centre of origin of several crops and has arguably the longest continuous history in the world of high intensity agriculture which has formed the basis of an agrarian society that continues to grow and develop in the present day. Greater than 60% of Indians depend directly or indirectly on agriculture for their livelihood. The subcontinent has harboured a population of >100m for over 300 years. However within the last 100 years the Indian population has increased over 4-fold, and within the last 50 years alone the increase has been over 2.5-fold. The demands this has placed on the land and resources available for agricultural production is

unprecedented in human history and it is remarkable that India has achieved self-sufficiency in food production although this has come at a cost: the increases in yield that have been achieved through the green revolution have plateaued and come at the expense of excessive utilization of groundwater resources. How and whether this will be sustained is a major question given the environmental and other costs and constraints: low soil fertility in many areas due to prolonged usage and excessive fertilizer application; limiting water availability (only 35% of the agricultural land is irrigated) and massive depletion of groundwater; lack of additional land area for agriculture; and climate change. While the level of Indian agricultural production before 1960 was precarious and there were imperatives to increase foodgrain production at all costs in order to achieve self-sufficiency and meet the national requirement, the present situation is one where India does have a food surplus in production terms. The total food productivity has increased 5-fold from 50 mt to about 250 mt in the last 60 years and India is now a major exporter of rice. In contrast to these achievements is the spectre of widespread hunger and particularly child hunger and malnutrition throughout the country. One third of the world's malnourished children are in India and parts of India have the highest percentage of malnourished children in the world. Child malnutrition has been acknowledged by the Prime Minister of India as a national shame.

The models of agriculture differ widely across the globe and within countries ranging from large scale industrial farming involving hundreds or thousands of acres, to small subsistence farms of an acre or less. In India the average farm size is 3.3 acres and the majority of farmers are small and marginal farmers. Environmental factors and the availability of resources also vary widely. It follows that principles and practices that may apply to one model may not be equally applicable in another case, it is also recognized that agricultural knowledge and practice has evolved over many millennia under diverse agro-ecological, climatic, and cultural conditions in different parts of the world. The

experience and knowledge that resides with farming communities locally and worldwide is clearly of relevance when considering policies and approaches for sustainable agriculture.

Agricultural Policy Considerations in Relation to Knowledge and Practices

Recognizing the enormous complexity and diversity of agriculture and agricultural practice across the world, as also the scope and seriousness of the challenges with regard to food production to feed a growing population on a sustainable basis worldwide, the World Bank and the Food and Agricultural Organization (FAO) initiated a consultative process at a global level to assess the status of knowledge related to agriculture across the world. This led to the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) which was carried out under the auspices of the World Bank and the UN and presented as a set of reports: one Global and five Sub-Global. These reports represented a large study extending over 4 years and involving over 400 experts across the globe. The aim of the IAASTD was to assess the entire state of information past and present on agriculture including traditional knowledge on agriculture and the impact this has had on reduction of hunger, improvement of rural livelihood and health, and equitable and sustainable development. The Synthesis Report of the IAASTD integrates the key findings from the Global and Sub-Global Assessments and focuses on different topics: bioenergy; biotechnology; climate change; human health; natural resource management; traditional knowledge and community based innovation; trade and markets; and women in agriculture. The reports outline broad policy issues and findings that came to light from the consultations in the context of the geographical area and/or topic. The IAASTD aims to respond to *"the widespread realization that despite significant scientific and technological achievements in our ability to increase agricultural productivity, we have been less attentive to some of the unintended social and environmental consequences"* (Executive Summary of the Synthesis Report (ESSR)). The ESSR emphasizes that the prevailing model being followed for

Agricultural Knowledge Science and Technology (AKST) which is based on maximizing productivity while externalizing costs (environmental and social) requires a fundamental shift in order to achieve development and sustainability goals and that such a *"shift would need to recognize and give increased importance to the multifunctionality of agriculture, accounting for the complexity of agricultural systems within diverse social and ecological contexts"* The ability of small household farms to provide food security as well and institutional changes that need to be made should be directed primarily at those who have been served least by previous AKST approaches, i.e. resource-poor farmers, women, and ethnic minorities." A Global Summary of the Report for Decision Makers (GSDM) lists 22 key findings, 8 of which are listed below:

- People have benefitted unevenly from yield increases (in agricultural production) across regions, in part because of different organizational capacities, sociocultural...factors, and institutional and policy environments.
- * Emphasis on increasing yields and productivity has in some cases had negative consequences on environmental sustainability.
- * An' increase and strengthening of AKST towards agroecological sciences will contribute to addressing environmental issues while maintaining and increasing productivity.
- * Greater and more effective involvement of women and use of their knowledge, skills and experience will advance progress towards sustainability and development goals and a strengthening of AKST to address gender issues will help achieve this.
- * Many of the challenges facing agriculture currently and in the future will require more innovative and integrated applications of existing knowledge, science, and technology (formal, traditional, and community-based) as well as new approaches for agricultural and

natural resource management.

- * Significant pro-poor progress requires creating opportunities for innovation and entrepreneurship which explicitly target resource poor farmers and rural labourers.
- Public policy, regulatory frameworks, and international agreements are critical to implementing more sustainable agricultural practices.

- * The choice of relevant approaches to adoption and implementation of agricultural innovation is crucial for achieving development and sustainable goals.

Some of the above findings find clear support in the Indian experience. For example it is well recognized that the benefits of the green revolution have extended mainly to resource rich farmers with access to good irrigation and have comparatively evaded resource poor and marginal farmers. The excessive use of groundwater for irrigation has also contributed to severe water crisis in several parts of the country. What is less well recognized is the role of policy environments and regulatory frameworks in defining and implementing sustainable agricultural practices. For example the GM regulatory guidelines in India do not specifically include socioeconomic or need-based assessments of products/technologies (taking into account alternatives), and the impact that these would have in the social context; the effect on resource poor farmers or sustainable agriculture is not specifically examined as part of the regulatory process. Majority of governments represented approved the ESSR and GSDM in 2008. .

Biotechnology and Agriculture

The term "biotechnology" covers a range of methods and approaches that are used for product and process development involving biological organisms that are of economic benefit. It includes methods such as microbial fermentation, plant and animal tissue culture, biofertiliser production, traditional cropping practices, and the use of advances in modern biology and genomics for marker assisted selection (MAS) in plant breeding. These methodologies do not involve, genetic engineering or gene transfer across the normal naturally occurring barriers that are imposed by sexual processes and breeding. GM biotechnologies on the other hand involve genetic engineering or gene transfer across the normal barriers imposed by breeding and sexual reproduction. The creation of genetically modified (GM) crops by such

methods is an example of GM biotechnology. Whereas many of the non-GM biotechnologies have been in existence for a long time and are widely accepted, the use of modern biotechnology for purposes such as making GM crops is more recent and has attracted considerable concern from a number of points of view with respect to safety for health and environment arising from uncontained growth and use of these crops directly or after processing for human consumption. The issues of concern discussed within the IAASTD included:

- * The adequacy of safety testing of GM crops and the regulatory frameworks
- * Whether GM crops would address the needs of most farmers and do so without harming others
- * Whether GM crops would make significant, contributions to small and subsistence agriculture

Usage of GM Crops

The deployment of GM crops has so far focused predominantly on reducing losses due to insect pests and weeds (Kaphengst et al., 2011), and also for engineering resistance to plant diseases, mainly viral disease. Other applications include increasing the tolerance of plants to drought and salinity, nutritional enhancement and modification, increased shelf life, and engineered male sterility. However, two types, of GM technologies have been predominantly deployed in crops worldwide. One is the engineering of resistance to insect pests by incorporating into the plant genome a gene from a soil bacterium (*Bacillus thuringiensis*), encoding a protein named Cry (for crystal; also called Bt protein) that is toxic to certain insects. The Cry protein is present in *B. thuringiensis* as an inactive precursor which is then activated in the environment of the insect gut when the insect ingests the bacterium, and binds to the surface of cells in the lining of the gut where it cause changes in

the cell surface and renders the cells vulnerable to invasion by bacteria present in the gut ultimately leading to infection and death of the insect by septicaemia (Broderick et al., 2006; Chen et al., 2007). Bt technology involves engineering plants for insect resistance by incorporating the gene for the toxin within the plant's genetic constitution, so that the plant becomes naturally resistant to the insect. The benefit of this is a reduced requirement for externally applied chemical pesticides most of which are toxic and cause environmental damage. Use of Bt technology in cotton has been shown to lead to significant reduction in usage of chemical pesticides (Qaim and Janvry, 2005; Krishna and Qaim, 2012). Other studies have shown that over time there is increased incidence of secondary pests in cotton leading to increased pesticide use and erosion of benefits of Bt (Wang et al., 2008; Lu et al., 2010). Out of 91 applications that are pending for field trials within the Indian regulatory system, 44 involve Bt in a wide range of crops: cotton, rice, maize, brinjal, cauliflower, cabbage, okra, pigeon pea, chickpea, and castor. The only GM crop currently deployed commercially in India is Bt-cotton which was introduced in India in 2002 and now occupies 95% of the total area under cotton (21 million acres). The other major usage of GM crops has been for herbicide tolerance (HT) which involves the use of a single broad-spectrum herbicide (most commonly glyphosate or else glufosinate) to be used to kill weeds while leaving the crop plant alive as it is genetically engineered to be resistant to the herbicide. The herbicide acts to inhibit an essential enzyme that is found in all plants and as a result is able to eliminate all weeds whereas most conventional herbicides are selective in their action and target a limited number of weeds. The resistant crop carries a transgene encoding an enzyme that is resistant to inhibition by the herbicide or else inactivates the herbicide. The use of HT technology allows more extensive application of the herbicide leading to more complete elimination of weeds without killing the crop. India does not currently have commercially deployed HT GM crops. Out of 91 applications before the GEAC, 17 were for herbicide tolerance in crops and included cotton, rice, maize, and wheat.

Six different Bt-cotton transgenics have been approved for commercial cultivation in India since 2002. Approval for Bt-brinjal was granted by the Indian regulatory body in 2009 but subsequently placed under moratorium by an executive decision Minister of Environment and Forests on the advice of experts from different parts of the world.

The spectrum of positions on the regulation of GM crops and public perceptions covers a wide range. One view is that the regulation of GM crops is excessive and that this constrains the pace and scope of the benefits that GM crop biotechnology is being able to bring to society which is being denied the full extent of its potential. According to this view the regulation of GM crops in its current state across the world including perhaps India, may be hindering the delivery of its benefits to the poor. An alternate view is that GM crop biotechnology being relatively recent, there is limited information on the safety of GM crops especially food safety and effects on the environment arising from long term and widespread consumption and release of GM crops. According to this view it would be prudent to carry out extensive testing, erring on the side of caution when it comes to evaluating GM crops for health and environmental safety. A third view which is not mutually exclusive with either of the two previous views is that concentration of Intellectual Property (IP) and resources for research on GM crops in the private sector is resulting in perverse and exploitative relationships of public institutions with the private sector in developing countries and that these are not successful at meeting development and sustainability goals. According to this view the control and driving of GM crop biotechnology by the private sector is affecting the ability to deploy it towards the public good in developing countries and perhaps in others as well.

GM technology comes with the promise of a number of benefits as well as associated risks with regard to health and environmental safety. These risks need to be clearly recognized and addressed in-order for GM products to gain societal acceptance and the potential benefits to be realized. Because of the broad scope of GM technology and the range of possible products, risk assessment would need to be considered on a casewise basis even

though there may be some, issue's that would be common to most cases. Part of the concerns about GM are influenced by the features of the two main GM technologies that have been deployed so far: Bt and HT. Both of these have drastic modes of action and act to kill the target species at high efficiency. The first is a toxin that is very efficient at killing certain types of insects. While it's lethal effect on short term consumption is specific to certain classes of insects (Soberon et al., 2009), there is health concern about the possibility that it may also have milder and less easily apparent effects on other animals and these effects may lead to adverse consequences at a lower frequency and/or over longer time. In the case of HT, the technology involves the combined use of a chemical herbicide and a GM plant. The herbicide is generally a broad spectrum herbicide meaning that it acts on a large number of plant species, so it kills all the plant species in the field, leaving only, the engineered GM plant to grow. One concern is that over-reliance on use of one or two herbicides in increased amounts over time as in the case of HT, leads to the emergence of herbicide resistant weeds and negatively impacts sustainability. Bt and HT technologies are discussed in more detail later in the report.

International Agreements and Instruments for Food Safety, Conservation, and Regulation:

The Codex Alimentarius Commission (CAC) implements the joint FAO/WHO Food Standards Programme and provides guidelines and standards for food safety. It develops guidance documents from time to time on issues related to food safety including foods derived from biotechnology. India became a member of the CAC in 1964.

The steps towards international agreements for the conservation and management of biodiversity began in the late 1980s within the United Nations Environment Programme which convened the Ad Hoc Working Group of Experts on Biological Diversity in 1988 followed by the Ad Hoc Working Group of Technical and Legal Experts in 1989. Discussions within this group led to

the text of the Convention on Biological Diversity.

Agenda 21 is a non-legally binding comprehensive plan for actions to be implemented globally, nationally, and locally by the UN, Governments, and other organizations in areas where human activity impacts the environment. *Agenda 21* was adopted by 178 Governments including India at the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992.

The Rio Declaration on Environment and Development is a statement of 27 principles for the purpose of guiding sustainable development across the world. Principle 15 states that:

"In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation," ■

India was one of the countries that adopted the Rio Declaration in 1992,

The Convention on Biological Diversity -- CBD (www.cbd.int) is a legally binding treaty that has three stated aims: the conservation of biological diversity, the sustainable use of the components of biological diversity, and the fair, and equitable sharing of the benefits arising out of the utilization of genetic resources. It comprises of 42 articles outlining principles and steps for the identification, conservation, and management of biodiversity that member countries would follow. 193 countries are parties to the CBD including India. The CBD also brings out guidance documents on issues related to biodiversity such as on risk assessment of LMOs (2012).

The Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity is an international agreement aimed at "ensuring an adequate level of protection in the safe transfer, handling, and use of living modified organisms (LMOs) resulting from modern biotechnology that

may have adverse effects on the conservation and sustainable use of biological diversity, also taking into account risks to human health, and specifically focusing on transboundary movements" (Article 1). Although enunciated in the context of transboundary movements, many of the terms also extend to conditions within a nation since handling and usage within the country impacts closely on transboundary movements. The Cartagena Protocol comprises 40 Articles covering handling, transfer, risk assessment, risk management, capacity building (in biosafety), public awareness and participation, socioeconomic considerations, and other issues. India signed the protocol in 2001 and became a party in 2003.

The CPB and CAC provide guiding principles for the biosafety of LMOs including GM crops. However the operationalization of GM regulation is left to individual countries. The CPB and CAC represent a minimum set of principles that national systems of biosafety regulation are expected to address in order to meet international obligations.

The Indian GMO Regulatory Structure:

The apex regulatory body for evaluation of GMOs/LMOs is the Genetic Engineering Appraisal Committee (GEAC) located in the Ministry of Environment and Forests (MoEF). The second arm of the regulatory body is the Review Committee on Genetic Manipulation (RCGM) located within the Department of Biotechnology (DBT) of the Ministry of Science and Technology. Examination of health safety and molecular characterization is the purview of RCGM and environmental safety comes under the overall purview of GEAC, however, RCGM also examines information on environmental safety. The responsibilities of RCGM also include review of applications for research projects involving recombinant DNA and animal experimentation. Both RCGM and GEAC have approximately 30 members. These include nominated representatives of government departments and agencies, researchers, and administrators.

The GEAC and RCGM/DBT have produced a number of documents and guidelines covering Recombinant DNA Safety, Guidelines for Research on Transgenic Plants, Guidelines and Standard Operating Procedures (SOPs) for Confined Field Trials, Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants (prepared by Indian Council of Medical Research). There is also a Guidance for Information / Data Generation and Documentation for Safety Assessment of Regulated Genetically Engineered Plants which is currently a draft document and describes the studies and tests to be carried out for safety assessment of GM plants.

Discussion of Recommendation of the Interim Report (IR) in the Context of the Terms of Reference for the TEC:

The TEC discusses below the recommendations made in the Interim Report with reference to the TOR so as to explain the basis for these recommendations.

TOR (d): To advise on whether specific conditions imposed by the regulatory agencies for Open Field Trials are adequate. If not, recommend what additional measures/safeguards are required to prevent potential risks to the environment.

The committee examined the conditions imposed on conducting Field Trials (FTs) by the regulatory agencies and was of the view that there were weaknesses. In particular, the practice of allowing the Applicant to choose the site for conducting the trials and leaving the onus on the Applicant to ensure conditions for safety introduces chances for violation of conditions safety. The TEC was informed that one of the criteria followed is that the land should be leased for at least three years. The TEC is of the clear view that this is not sufficient and sites should be either in control of the regulator or else part of a permanent facility that is certified and periodically examined by the regulator on land that is owned by the Applicant/Tester. The regulator should establish and certify such sites either in ICAR institutes or State Agricultural Universities (SAUs) with suitable isolation and restricted access (walled

area) and appropriate facilities for conducting the field trials, associated biosafety tests, and facilities for disposal (incineration of plant material) etc,

The restriction of FTs to well confined areas that are under the control of the regulator and paying close attention to operation would substantially address

the concerns regarding contamination and environmental risk during trials. The development of designated and well confined sites for FTs, their certification, and rigorous implementation of operating procedures at these sites for FTs is an important requirement that needs to be put in place. On the above basis as well as addressing structural and functional weaknesses in evaluation (see TOR (f) below) the TEC had recommended that FT's be discontinued until the required conditions were met.

TOR (a): To review and recommend the nature of (and)sequencing of. risk assessment (environment and health safety) studies that need to be done for all GM crops before they are released into the environment.

The TEC considered the nature of and sequence of risk assessment studies currently being done. These are given in the draft document on Guidance for Information/Data Generation and Documentation for Safety Assessment of Regulated Genetically Engineered Plants. The TEC noted that Post Release Monitoring (PRM) is also an important aspect of environmental safety as well as health safety (if the plant is consumed as food) and this has not received adequate attention in the regulatory system (1R: p3, 9) or in practice.

The TEC also noted the importance of need and socioeconomic impact assessment of GM products as one of the criteria that should be applied in the evaluation at an early stage (IR: p3, Annexure 1) and had also suggested broadening of expertise in this context (IR: p11). it is ironic that whereas the importance of socioeconomic considerations, sustainability, and development goals is well recognized in the international agreements that India has signed/accepted (e.g. CBD, CPB,) these criteria do not specifically figure in its own national regulation particularly keeping in mind that meeting the development and sustainability goals is highly relevant in the Indian context. This deficiency needs to be recognized and corrected.

The TEC had also raised and given reasons for the need for additional tests that are not presently being done but which need to be introduced:

- (i) longterm feeding studies for assesment of chronic and intergeneration toxicity in small animals (p7, 12)

The dimensions of health risk assessment can be visualized from the fact that we are not merely exposed by reared on food. Thus all the stages of human life cycle including the most vulnerable period, the early gestation period of pregnancy need to be kept in mind. Therefore there is a need to test for the possibility of chronic effects as well as transgenerational effects in small animals. Such effects have been observed in the case of certain drugs There has also been recent evidence in the literature (after submission of the IR) that points to the possibility of chronic effects being observed at dosages where no effect was seen for sub-chronic exposure. These studies bring out the need for inclusion of chronic exposure testing for food safety (Seralini et al., 2012). (if)

- (ii) Genomewide expression analysis in the toxicity studies to screen for possible unintended effects on host physiology. For example certain types of GM products (those involving RNAi or antisense mediated knockdown of an endogenous gene) have the possibility of having unintended effects due to off-target knockdown of other genes in the plant (Singh et al., 2011) as well as transfer of small RNAs to the host that might alter host gene expression and physiology (Zhang et al., 2012). This study needs to be included to give a more detailed picture of a possible difference in effect of GM food compared to its non-GM counterpart.

With regard to herbicide tolerance, the TEC had expressed concern that HT technology may not be suitable in the Indian socioeconomic context because of

a possible impact of extensive use of broad spectrum herbicides on the environment and biodiversity and also that the technology was more suitable for large farm size of hundreds of acres whereas the average farm size in India is 3.3 acres. The TEC had recommended a moratorium on field trials of HT crops until the issue had been examined by an independent committee.

In the case of crops of Indian origin or diversity the TEC had recommended that transgenics not be allowed for field trials as the deployment of transgenics commercially under open conditions (once they had cleared the tests for confined field trials and been approved for release) would adversely affect the diversity of these crops which represents an important cultural heritage and special measures should be taken to preserve it as much as possible.

In considering the; sequence of tests, an early step is the identification of transgenic plants or Events that show by preliminary examination, characteristics that would be desirable and appear free of growth defects or weaknesses from among the large number of transgenics that are initially obtained. The Event Selections form a major first filter to identify candidates that would be pursued in further performance and safety tests. The TEC recommended that Event Selections be undertaken in the greenhouse if possible. If not possible (see Tor (c) below), they may be undertaken at a designated location that has been certified by the regulatory agency under contained (restricted access) conditions i.e. those conditions under which confined field trials are to be performed (TOR d).

TOR (b): To recommend the sequencing of these tests in order to specify the point at which environmental release though Open Field Trials can be permitted.

The TEC was of the view that there should be requirement of some basic information on biosafety prior to FTs (IR: p6 last para) and also recommended that some experimental tests should be done prior to emergence from containment in the laboratory or greenhouse and suggested possible examples (IR: p5-6). These can be considered on a casewise basis in discussion with the regulator. In any case, it should be possible to do laboratory based tests such as for acute toxicity on the purified protein

- since this would not require the transgenic event as well as others such as test for possible allergenicity and toxicity based on bioinformatics analysis (Guidance for Information/Data Generation and Documentation for Safety Assessment or Regulated, Genetically Engineered (GE) Plants : p5, 11). it should also be possible to do the basic molecular studies with to copy number of the insertion and sequence of the protein encoded by the insert copy, as part of or immediately after Event Selection and before FTs, since this would require only limited amount of plant material (few grams for each plant being tested).

TOR (c) To advise on whether a proper evaluation of the genetically engineered crop/plants is scientifically tenable in the green house conditions and whether it is possible to replicate the conditions for testing under different agro ecological regions and seasons in greenhouse?

Plants being sessile organisms, have evolved numerous responses and adaptations to changes in the environment (light, water, humidity, temperature, wind, seasons, and soil quality). The properties of the plant will depend upon the complex relationship between how these factors affect plant growth, development, and response. The growth and health of the plant is thus very sensitive to these factors. In general, it is very difficult to replicate in the greenhouse, the conditions that would apply in the outside environment Ideally it would be desirable to do event selections and as many tests as possible under containment within the greenhouse, however, the TEC left it on a casewise basis for the Applicants consultation with the regulator to do

event selections either in the greenhouse or under confined conditions outside the greenhouse (IR: p5-6, p8 para 1).

TOR (e): Examine the feasibility of prescribing validated protocols and active testing for contamination at a level that would preclude any escaped material from causing an adverse effect on the environment.

The TEC had not specifically addressed this issue in the IR.

TOR (f): To advise on whether institutions/laboratories in India have the state-of-art testing facilities and professional expertise to conduct various biosafety tests and recommend mechanism to strengthen the same. If no such

institutions are available in India, recommend setting up an independent testing laboratory/institution.

To examine the professional expertise in biosafety testing, the TEC examined the data for various biosafety tests from the safety dossiers for Bt-cotton and Bt-brinjal some of which were available in the public domain at the website of the regulator (igmoris.nic.in). The dossier for Bt-brinjal had been examined by others including international experts who had commented on the data and pointed out certain concerns. The TEC examined these and also found other instances where there were significant differences in biological indicators between Bt and control samples (IR: p8) in the case of cotton. These are pointed out in more specific terms in the final report (Section on Examination/Study of the safety dossiers).

Of greater concern was the finding that these problems had gone unnoticed and unaddressed in the course of the regulatory process leading to approval. This led the TEC to consider the examination process and it became apparent that the scrutiny of the biosafety information was being done by the committee of the regulatory body which lacked full-time qualified personnel for the purpose.

Taken together these observations led the TEC to point out the need for a dedicated team of scientists for examination of safety data. The TEC also pointed out conflict of interest issues with regard to location of RCGM within DBT when the latter has a mandate of promoting biotechnology. Having come across examples of problematic data the TEC's conclusion was that the data as a whole did not establish health safety for Bt-cotton and Bt-brinjal and left unanswered questions about the overall safety of Bt in food crops. This led the TEC to recommend a ten-year moratorium for Bt in food crops giving specific reasons (IR: p14-15). This issue has been revisited for the purpose of the Final Report.

The TEC also recommended reexamination of the safety data of the

approved applications to ensure that all the biosafety issues have been addressed. Reexamination of the data is not uncommon and the TEC came across instances of this in other regulatory systems as well.

Deliberations of the TEC Following Submission of the Interim Report

Following submission of the Interim Report the TEC received the order of the Court appointing a sixth member to the TEC and directing the TEC to submit its final report. The TEC then held eight meetings in New Delhi between December 2012 and April 2013 where extensive discussion and exchange of views took place including discussions with members of the National Academy of Agricultural Sciences, and inter-departmental group comprising the Secretaries from the Department of Agriculture and ICAR, senior officials from MoEF and DBT, senior researchers from ICAR institutes, and a separate meeting with the Secretary, DBT. Members of the TEC also met the Secretary, State Agriculture Department of Andhra Pradesh, and visited a research facility of a multinational research company in A.P. carrying out research on GM crops to examine the research facilities. The TEC also received written submissions from Prof. Deepak Pental, ABLE, National Seed Association of India, and others. Several of the respondents particularly from the side of Gol expressed concerns about the recommendations in the IR relating to moratoria on Bt food crops, HT, and restriction on GM in crops of Indian origin/diversity. These points were further discussed within the TEC. The TEC then held meetings at National Institute of Nutrition, Hyderabad over April 18 to May 10 for the purpose of preparing the Final Report.

Issues that were discussed in the course of deliberations by the TEC included the following:

- i) The TEC discussed the need for a systematic process of hazard identification, hazard characterization, exposure characterization, as part of an overall process of risk assessment to be carried out on a casewise basis so

as to address all the issues of food safety as given in the CAC guidance for food safety and environmental safety as given in the CPB guidance. It would be important to identify and specifically address all the safety concerns rather than selectively identify the recommendations to pursue by following a set of tests laid down in the guidance. This would establish a standard that the risk assessment meets the requirement of the CAC and CPB so as to comprehensively cover the issues rather than do so partially and would provide assurance for example for the purpose of trade or other cases of transboundary movement. If the compliance is partial, based on execution of a limited set of tests, then it can create uncertainties at various levels. Such an exercise would require systematic hazard identification at the beginning of the risk assessment. The overall process would require a thorough understanding and possibly training in the practice of risk assessment on the part of the examiner and regulator as well as the applicant.

ii) The significance and value of chronic toxicity testing which is currently not being done for GM regulation either in India or elsewhere on a regular basis, the rationale being to address the possibility that a particular toxic effect could be seen on chronic exposure which is not apparent in acute and sub-chronic studies. Possibilities could include transgenerational effects by analogy to what has been seen in the case of endocrine disruptors where effects are seen only on the next generation (Endocrine Disruptors and Child Health, WHO Report, 2012) in development, or even behaviour. On these grounds the TEC considered it appropriate that longterm and transgeneration testing of food safety on small animals be included as one of the tests. The TEC learnt that the issue of chronic toxicity testing has also been under discussion in GEAC. The TEC was of the view that this would not place an undue burden on the applicant since the generation time for mice and rats is short (2-3 months) and it should be possible to do the tests within 1-2 years in parallel with other tests. A recent study on chronic exposure of rats to herbicide (glyphosate) or HT plants (concluded that the animals had a higher chance of developing tumours than control animals and that treated animals

developed tumours earlier than did the controls (Seralini et al., 2012). The study drew criticism from certain circles but was considered valid by others. However it did make a case for the need to include chronic toxicity tests as one of the dimensions for food safety of GM crops. There is now evidence that the adjuvants used in herbicide formulations comprising surfactants and solvents to promote penetration of the herbicide into the plant are toxic more so than glyphosate itself (Richard et al., 2005; Mesnage et al., 2012). These results show that herbicide formulations need to be assayed for toxicity rather than just the isolated herbicide

iii) Another issue of discussion was the possibility of unintended effects arising from altered regulation of plant or host genes by processing of novel RNA species produced from the insertion(s) in the transgenic plant. A recent study (Zhang et al., 2012) found that small regulatory RNAs can be transferred from the food to the host bloodstream where there is a possibility of their acting to regulate host genes. If novel RNAs are made in the food and reach the host bloodstream then this raises the possibility of new targets in the host and different effects on the host gene expression physiology (Heinemann et al., 2013). Changes in host gene expression can be examined by a combination of bioinformatics analysis with whole genome expression profiling of the host and these tests can be done on model test animals (mice or rats).

iv) The TEC discussed the need for designation of specific sites for confined field trials (corresponding to BRL1 and BRLII in the guidelines) and that these would need to be in ICAR institutes and State Agricultural Universities. The role of ICAR in providing support to the regulatory body in conducting trials was also discussed.

v) So as to establish full performance testing of any GM material following approval by the regulatory body, before it is released commercially, the TEC discussed whether release of such material should be on the basis of trials conducted by the All India Coordinated Research Programme (AICRP) rather than an event based approval which is currently the practice.

vi) The TEC was also informed by the Secretary, DoA that it will not be possible to segregate GM from non-GM material during the overall process of collection, handling, and storage in India. This would have serious implications when it comes to labeling of GM and non-GM food. The Indian legislation requires labeling of packaged food. There are also serious trade implications with regard to export of food and grain and how these would be affected if India starts to grow GM food crops. If there are concerns or even consumer unease about the accuracy of GM labeling then it could have an adverse effect on export. There are currently 18 food crop species for which applications for field trials have been received in the Indian system: cauliflower, cabbage, corn, rice, wheat, tomato, groundnut, potato, sorghum, okra, brinjal, mustard, papaya, watermelon, sugarcane. For example the TEC was informed that GM rice trials are not being permitted in areas where basmati rice is grown. However, if GM rice were to be approved for commercial release then it is unclear how stringently it would be possible to enforce such control at the production level. Moreover it could also impact export of non-GM rice. The total annual export of rice from India is approximately Rs. 12,000 crores.

vii) A consequence of the inability to segregate GM and non-GM food products and the uncertainties in labeling would also be that it will be very difficult to carry out postrelease monitoring for health effects if this is deemed at all necessary in future, because of the difficulties in being able to separate GM users from non-GM users. It may be possible to do this in an isolation study but this would probably be unrealistic in most cases.

viii) The growth of GM crops in India would impact organic food producers and given the difficulties in segregation of GM and non-GM food as well as small size and fragmented nature of the plots it would be very difficult to meet the criteria for organic food. Since the organic food consumer and organic market requires strict adherence to requirements for organic certification, and organic food importers often closely examine the conditions under which

organic food is being grown, any concerns about contamination could lead to an adverse impact and loss of markets to organic food producers. The TEC received a submission from organic food representatives expressing their concerns.

ix) The TEC also discussed the advisability of India getting into growing such a large number of GM crops at once, given the uncertainties that remain regarding health and environmental safety, weaknesses in the regulatory system, effects on non-GM growers, issues regarding segregation and labeling of GM products, and the position that it will not be possible to segregate GM and non-GM products as well as economic and trade implications. Most countries such as China and those in Europe are approaching the issue with a fair amount of caution. Even where regulatory approvals have been granted as in Europe by EFSA, several countries have refused to permit growth for various reasons. Some sort of prioritization needs to be brought into consideration of GM crops in the Indian context. For example in the case of oilseeds where there is a large requirement and a high import (about Rs. 50,000 cr annually) a case can be made, and since oil would be used after processing and refinement, the food, safety concerns would be less than if the GM crop was being directly consumed. In the US, most of the maize used for human consumption is after processing and because there is no labeling it is hard to say how much GM maize is being directly used as food for human consumption.

x) Another issue related to the way tests for toxicity and data analysis are being done and statistical criteria being employed. The TEC found instances of wide variation in the starting parameters and evidence of nonrandomness between the control and test populations which affect the validity of any conclusions being drawn. The test and control populations should meet the criteria of being drawn from the same population and the mean and degree of variation of the relevant parameters between the two populations must be

comparable. If they are not then the statistical tests need to be modified. Depending upon the test, the sensitivity and degree of confidence required, and from the variation present in the population based on historical information, the number of animals in the test and control population should be chosen. The question of whether a statistically significant difference that is observed is biologically significant or not cannot be considered after the test is done. The TEC came across instances where this situation is encountered and was also informed that this has come up in the course of discussions with members of the regulatory body. If this happens, the test needs to be repeated with more animals so as to give more conclusive information.

xi) The TEC discussed the possibility of the Indian regulatory body undertaking a collaboration with the regulatory body of a country which has an established and reputed regulatory system through workshops and training of personnel as well as exchanges. Norway is one country that has experience in integrating socio-economic considerations into GMO regulation as the Norwegian Gene Technology Act includes the criteria of sustainable development and societal utility (Myhr and Rosendal, 2009). The Norwegian Agency for Development Cooperation (Norad) has partnered with a number of developing countries for successful programmes in biosafety capacity building so they appear to have established courses and training programmes in place.

Examination/Study of the Safety Dossiers

In order to study the information being provided by applicants/developers as part of the overall safety assessment process, the TEC has examined/studied the data from dossiers provided by MoEF to the TEC, as well that available from the website (igmoris.nic.in) including the events approved for commercial cultivation for which the information collection and examination process is complete..In examining the data, the TEC has sought to obtain a general overview of the extent and quality of information that is being provided as well as the overall assessment that is being done in

relation to safety. A second purpose is to get an idea of the type of products that are being developed. The TEC would like to make clear that this examination does not represent a detailed review of the safety dossiers even though certain examples may be mentioned. These are meant to be illustrative and not comprehensive. The overall purpose is to identify areas that need to be strengthened and to suggest improvements.

Molecular data:

The molecular information asked for in the, guidance document comprises description of the gene product and its function; description of the trait; characterization and description of the inserted genetic material; number of insertion sites; organization of the inserted genetic material at each insertion site including copy number; whether inserted copies are complete or partial ; whether rearrangement have occurred upon integration; sequence data of the inserted material and of the flanking regions bordering the site of insertion; identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.

Regarding the first six cotton events that were approved, the TEC finds that the quality and extent of information was variable. In the case of MON15985, the characterization of the insertion has been done in detail including determination of copy number of the insert and tests for the presence of partial copies along with controls for sensitivity of detection (MON15985 dossier vol 2: p79-84; p89-90). Insert copy number determination is a critical component in the characterization of an event and should always be done with high accuracy and confidence. The insert should as far as possible be present in single copy as presence of additional copies including partial copies increases the likelihood of instability of gene expression arising from interaction between the two gene copies due to the possibility of homology based gene silencing which can result in reduced expression of the

trait in subsequent generations (Fagard and Vaucheret, 2000). For this reason copy number characterization should be done early in the risk assessment process so as to identify single copy events and eliminate others.

As it turns out MON15985 and its progenitor MON531 both have a second partial copy of the Cry 1.Ac gene inserted close to the full length insert. This would normally be a cause for concern due to the possibility of gene silencing leading to reduced expression and instability of the trait. This point should have been noted and raised in the course of examination. The onus would then be on the developer to provide compelling evidence in favour of stable expression of the trait, and this evidence would be expected to be part of the dossier (which it is not). In the case of both MON531 and MON15985, the events have been extensively deployed after testing in the USA and elsewhere and it is possible that this information exists with the developer (Mahyco/Monsanto), but unless asked for it may not be provided. Much of the safety data for MON531 and MON15985 including the molecular data are part of submissions that have been prepared in the USA for that regulatory system and resubmitted in the Indian applications. Unless comprehensive information is insisted upon in the Indian system, there are likely to be shortfalls in the review process and there is a risk of the message going out that the review process is less than stringent. The assessment process thus needs to be rigorous so as to establish an expectation of high scientific and technical standards.

In the case of some of the other applications that were approved, the information has been provided in very cursory form. For example in the case of GFM CryIA (Nath Seeds) the molecular data given to the TEC for examination comprised the sequence of the Cry1 protein, the map of the plasmid, the sequence of the site of insertion of the transgene, and the test for event-specific detection. The gene is stated to be a hybrid of CryIAb and CryIAc. The sequence of the protein is provided as a printout from a slide presentation and is very difficult to read. Data showing an event-specific protocol has been presented, however it does not include the sensitivity at which it works.

In the case of JKC-738 (JK Agrigenetics) too the information has been

given in cursory form as a printout of a slide presentation. The information provided to the TEC comprised references for the CryIAC gene, schematic diagram of the vector and construct, brief points of the history and partners along with Southern analysis of the event on the basis of which it is inferred to contain a single copy of the gene which followed Mendelian inheritance, statement of the transformation method, Western blot data for CryIAC protein expression in the plant, and Southern data claiming distinctness of the CryIAC event from MON531. The Southern analysis for determining copy number of the insertion does present evidence for presence of the CryIAC gene in single copy (Event 1), however the probe used is an internal fragment of CryIAC which does not rule out the presence of other parts of the T-DNA in more than one copy which can also influence expression of the trait. The same concern applies to characterization of the copy number of the Cry1 EC T-DNA (Event 24). More Southern analysis including probing with the entire T-DNA region as well as parts of the T-DNA along with including sensitivity controls is required before it can be reliably concluded that the insert is present in single copy. Sensitivity controls have not been included in the Southern analysis that is currently present in the safety dossier. The LOD 0.01% protocol for Event 1 that has been developed uses Real Time PCR and does effectively and reproducibly detect the presence of the CryIAC. However, the primers are directed towards sequences within CryIAC, and aimed at detection of CryIAC and not the specific event. The protocol is therefore not an event-specific protocol and would detect the presence of CryIAC in other transgenic events as well. The LOD 0.01% protocol for Event 24 (Cry1 EC) also works efficiently, however it is not clear whether this too is gene-specific or event-specific. The TEC has not been provided with the data demonstrating the inheritance pattern of the trait in subsequent generations.

In the case of the NHH44 BNBt application (CICR, Nagpur), the TEC has been shown information relating to description of the plant and the gene, brief description of the transformation procedure, nucleotide sequence of CryIAC, map of the the vector (two schematic diagrams of pBINBtS are given

on p7 and p11 respectively and the orientation of the insert is different in the two), data for characterization of the inserted gene in the plant comprising Southern analysis to establish copy number, and sequencing of the insertion site. The Southern analysis does not include sensitivity controls and hence it is difficult to rule out the presence of additional copies of insertions including partial copies. An event-specific protocol has been developed based on sequence information of one end of the insertion.

For the MLS9124 event (Metahelix), the molecular documentation provided to the TEC was the description of the plant material, the map of the vector pMH82 containing the CryIC gene, sequence of the protein, sequence of the T-DNA region of pMH82, information on insect range, and the protocol for event-specific detection at LOD 0.01%. The LOD 0.01% protocol provided in the dossier is construct/gene specific for CryIC (the reagents are designed to detect CryIC) and cannot rigorously be said to be event-specific as another event harbouring the same CryIC gene would also give a positive test. The applicants have also provided another protocol for LOD 0.01% using T-DNA primers, however this may be even less specific in practice as T-DNA regions are present in all transgenics made using *Agrobacterium* and there are chances that other transgenics may also give a positive test with similar results. An event-specific protocol should be based on specific information for the site of insertion in genomic DNA and incorporate primers that would amplify the junction region comprising sequences from the insertion and the genomic DNA. To clarify this point, the TEC wrote to the applicants who replied and sent information to the effect that they have developed such event-specific tests for MLS9124. However this information does not appear to be present in the dossier (at least the TEC was not provided with this information from the dossier) based on which the event was approved. The event therefore appears to have been approved with a reduced interpretation of the requirement for an event-specific protocol. The distinction between these protocols as the basis for approval was discussed by members of the TEC with a member of the regulatory body and the TEC was informed that construct-based tests are appropriate and sufficient for the purpose. The TEC is not in agreement with this view and feels that the requirement for an event-

specific protocol (as per the SC order dt. 8/05/07 in the present case) should be strictly adhered to.

The Bt-brinjal molecular data is presented in Chapter 3 of Toxicity and Allergenicity Studies (vol 1). The description of the plant material and method for making transgenic plants, description of the vector and the genes and genetic elements present in the construct used for transformation, the source and sequence information for the genes, and characterization of the inserted DNA are presented. In characterizing the inserted DNA with respect to copy number of the insert, Southern analysis has been performed for which the CryIAC gene has been used as a probe. However, the design of the vector pMON10518 is such that only one border sequence (RB) is present in the vector from which DNA transfer to the plant cell initiates and continues (unless terminated by some means) so that the entire plasmid is transferred. Use of a CryIAC gene as a probe does not provide information on the copy number status of the rest of pMON10518 so the conclusion that the "plants have only a single insert" is not justified by the data provided. What is needed is to use the entire plasmid as a probe and show that the expected size bands are present in single copy along with sensitivity controls in the form of exogenously added fragments of the plasmid in quantities that would correspond to a 0.5 genome equivalent of DNA.

The TEC has noted that in several of the cases examined, the characterization of the inserted DNA is limited and insufficient for comprehensively addressing the issues related to regulatory approval. For example very few if any of the applicants have undertaken to determine the precise sequence of the inserted gene and whether it encodes a protein that is identical to that in the starting construct or has changed in any way as a result of mutation or rearrangement which may occur in the course of insertion of the DNA in the plant genome (para 31 Codex CAC/GL 45-2003, 2003; Wilson et al., 2006):

"Information should be provided on the DNA insertions into the plant genome; this should include:the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region, sufficient to identify any substances expressed as a consequence of the inserted material, or, where more appropriate^ other information such as analysis of transcripts or expression products to identify any new substances that may be present in the food; and identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins." (CAC/GL 45-2003)

Overall the quality of information in several of the applications is far below what would be expected and required for rigorous evaluation by a regulatory body and is unlikely to meet international regulatory guidelines. For example the Codex Alimentarius requires that sensitivity of all analytical methods should be documented (Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants. Section 3, para20, CAC/GL 45-2003). The majority of applications have not used sensitivity controls in determining copy number of the inserted DNA. The examination of the molecular data by the TEC clearly pointed to the need for the submissions to be scrutinized in detail by dedicated independent scientists with expertise in both subject area and biosafety and not in a committee mode.

Health Safety Data: in considering the health safety and toxicology tests the TEC has examined from the approved Bt cotton events and that of Bt brinjal, the files relating to acute toxicity and sub-chronic toxicity on rodents and feeding and lactation studies on goats, and cows. Several common issues have emerged from this examination:

1. In several cases the methodology and results are not clearly reported.
2. The statistics have not been clearly described and except in a few cases the detailed statistical evaluation and testing is absent, in some cases the statistical treatments were inappropriate.
3. Often, the sample sizes even when meeting the minimum number given in the guidelines are insufficient to be able to find physiological/clinical differences that could be significant. In some cases the minimum number given in the guidelines is not met.
4. In certain cases, the differences between groups were described as being insignificant whereas they were statistically significant.
5. Often, the samples were not normal and homogenous and the distribution of test animals among treatment and control groups was not at random. In this case the statistical treatment would need to be appropriate.

Some of the examples are discussed below:

Examples of differences in haematological parameters, serum enzymes, and organ status that the TEC came across in the dossiers:

1. Mahyco CrylAc (HD73) MON531 dossier Vol 1:

Acute toxicity in mice:

Appendix 1, Table 6: 3/30 males • in the Bt group showed abnormal liver colouration as compared to 0/20 in non-Bt group.

This is not statistically significant but worth noting.

Subchronic 90-day study in goats:

In table 21 Neutrophil counts shows significant difference between Treatment Groups I (Bt) and II (non-Bt) ($p < 0.01$).

In Table 23 Lymphocyte count shows significant difference between Treatment groups I and II ($p < 0.02$).

Sample size for one or more of the groups In Tables 17 to 42 is 3-5 which is less than the minimum stipulated number.

In Tables 37 and 38 (p171), Serum Alkaline Phosphatase values show very large variations to start with (in one case the S.D. exceeds the mean) which makes it not possible to detect any difference in the experiment.

2. UAS Dharwad / CICR Nagpur NHH 44 Bt Cotton:

Acute toxicity: ACUTE ORAL TOXICITY STUDY IN RATS WITH NHH44 BT COTTON SEEDS (SHRIRAM, Tox 355a, 000041398, 23. 03. 2007)

Methodology issues:

- (i) , There is no evidence in the dossier of the identity of the test material having been confirmed. On p9 it is stated that *"the sponsor is responsible for the necessary characterization and evaluations of the test substance. The details of the test substance provided by the sponsor are as follows: ..."* it seems to have been accepted at face value by the testing laboratory (and the regulator) without any independent testing whether Bt transgenic cotton is indeed that, and the nontransgenic cotton is in fact non-transgenic. The labeling by the sponsor has been taken on faith.
- (ii) p10: details of experimental design have not been provided.

- (iii) p11: Animals were caged in groups of 5 whereas animals should be housed two per cage or individually. Justification for housing 5 animals per cage has not been provided. In this case housing several animals per cage can create differences in access to and consumption of food due to competition and behavioural factors. p11: Diet: "*Water and standard rat pelleted feed (Amrut Feeds Ltd) was freely available to the experimental animals*". There is no analysis of animal feed, its composition, quality, nutritional status, contaminant analysis or for microbial infestation: For batch to batch quality assurance, at least the proximate analysis should have been undertaken. The analysis of animal feed is an essential component of the toxicity studies. There is no statement about the quality of water, as well.

These are important requirements of toxicity studies which were ignored by the Expert Committees or the Regulators, who evaluated the dossiers.

- (iv) The copy of the dossier on the website has no signatures of the person(s) responsible for the study.
- (v) The Summary (p6) states that ""Under the conditions of the study , the single oral administration of *NHH 44 Bt-Cotton seeds and NHH44 Non Bt - Cotton seeds at the dose level of 5000 mg/kg body weight* to Wistar rats did not induce any treatment related observable toxic effects, *when compared to its control group of animals treated corn oil (vehicle) only.*" The relevant comparison should be between the transgenic and isogenic nontransgenic as a control. The inclusion of a vehicle control is inappropriate to the analysis.
- (vi) p13: Method of administration - "... administered with *NHH 44 Bt cotton seeds orally at the dose of 5000 mg/kg body weight with the*

help of a metallic cannula attached with tuberculin syringe." The cotton seed must have been powdered for this purpose, however that has not been indicated in the dossier. This is not acceptable at face value. If any processing of the cotton seeds was involved this needs to be mentioned. Furthermore mixing the cotton seed with oil and force feeding it to the rat through the canula does not simulate or reflect the normal mode of human consumption. Real life simulation of exposure is an essential component of health risk assessment. (vii) p22: Summary of observation

Table 1.03 Summary of Observations - Males and Females

Groups	Clinical observations	Necroscopy
Control	No toxic signs or symptoms was noticed	No noteworthy findings
Bt Cotton	No treatment related toxic signs or symptoms was noticed	No noteworthy findings
Non Bt Cotton	No treatment related toxic signs or symptoms was noticed	No noteworthy findings

There can not be a more casual approach of reporting the observations or results of a study. Nowhere in the dossier is there any mention of the clinical signs for which the animals were observed. What is considered noteworthy has not been defined.

(viii) Body weights and growth rates: The body weights have been presented in percentile forms which basically indicate the relative growth rates of the animals in different groups. The age of the animals at the start of the experiment is indicated on piO as 6-7 weeks. The original body weights of day of treatment designated as 0 day and then on the last day of the study (14th day) have also been presented revealing certain unusual observations which are difficult to accept in biology.

Table 1.04 (p23) & Table 1.08 (p27)

Mean			
14			
Group	Vehicle control	Non-Bt cotton	Bt cotton
Males	107.57±0.43	106.66±1.05	107.44±0.46
Females	107.22±0.94	107.01±0.77	107.22±0.25

The growth of animals in 14 days was similar in all the groups across gender, males and females being comparable.

Table 1.08, (p27)_s Average Weekly Body Weights of Rats

	Mean Body Weights (g)		
	on Bt		
Males	171.40±7.56	171.60±5.07	171.20±6.83
Females	169.6±7.60	171.14±6.58	174.20±5.63

p27: Table 1.08, Average Weekly Body Weights of Rats

	Mean Body Weights (g)		
Males	184.40±8.29	184.40±6.06	182.60±6.80
Females	181.80±6.57	183.80±7.46	186.40±5.12

The animals with no gender bias at about 7-8 weeks of age and at 9-10 weeks (6-7 week old animals were acclimatized for a week followed by a 14 day study period) had harmoniously comparable body weights with females gaining as much as males. The overall weight gain in two weeks, about a gm per day is rather low. Animals at this age should grow above 200 gm in body weights. Similar results indicating equivalent growth in body weights were obtained in the sub-chronic oral toxicity study in rats: /

Sub-chronic Oral Toxicity Study in Rats with NHH44 Bt Cotton Seeds

(SHRIRAM, Tox 355b, 000046990, 18. 05, 2007):

p31-36: Mean body weights from Tables 1.11-1.16

	Mean Body weights (g) Week 0					
	Vehicle	Non-Bt	Bt	Vehicle		
Males	132. 9	132. 5	131. 3	229. 8	231. 3	233.2 ±3.65
Females	131. 6	132.3 ±4.00	130.4 ±4.17	228.1 ±4.09	231. 9	231.5 ±4.09

(A 6-8 acclimation for

it is for the first time we come across biological gender equality in terms of body weights and growth rates from the age of 6-8 weeks onwards to 20-22 weeks of age of the rats. To our knowledge, it is not a common observation in any strain of rat (Figure 1). The same gender equality is also seen in the Metahelix MLS 9124 rat 90-day subchronic study (see below). It is very difficult to accept these data.

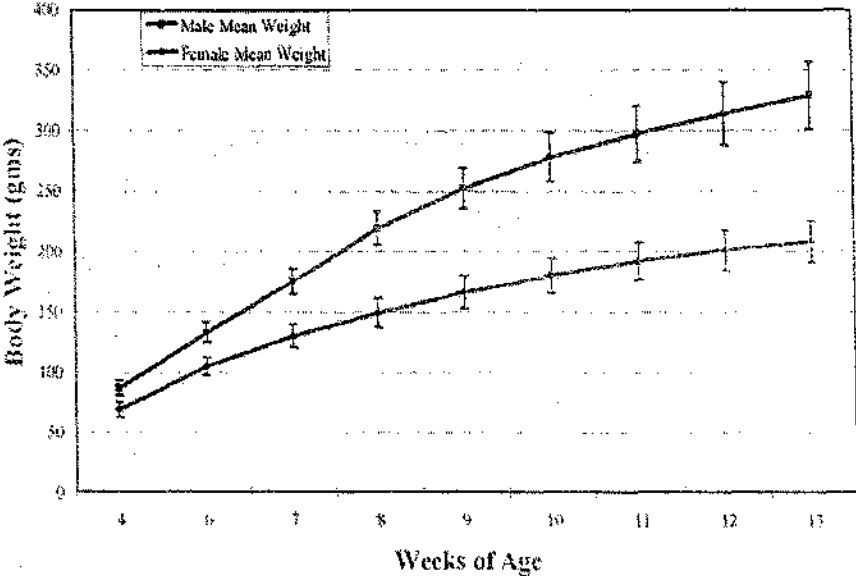
NHH 44 Bt Rat Acute Toxicity dossier:

p31-32: Mean Hematological Data from Table 2.0 and 2. 02

Test	Sex	Non-Bt	Bt Cotton	Significance (t-test)
Neutrophil count	M	18.4 ±2.60	15.2 ±2.49	ns
	F	18.0 ±2.65	13.8 ±2.28	p < 0.05

Global Alliance for Laboratory Animal Standardization (GALAS)

Wistar Hannover GALAS Rat 13 Week Growth Curve



National Laboratory Animal Centre, Mahidol University, Thailand

Growth rate of Wistar Rat (Gen. 47)

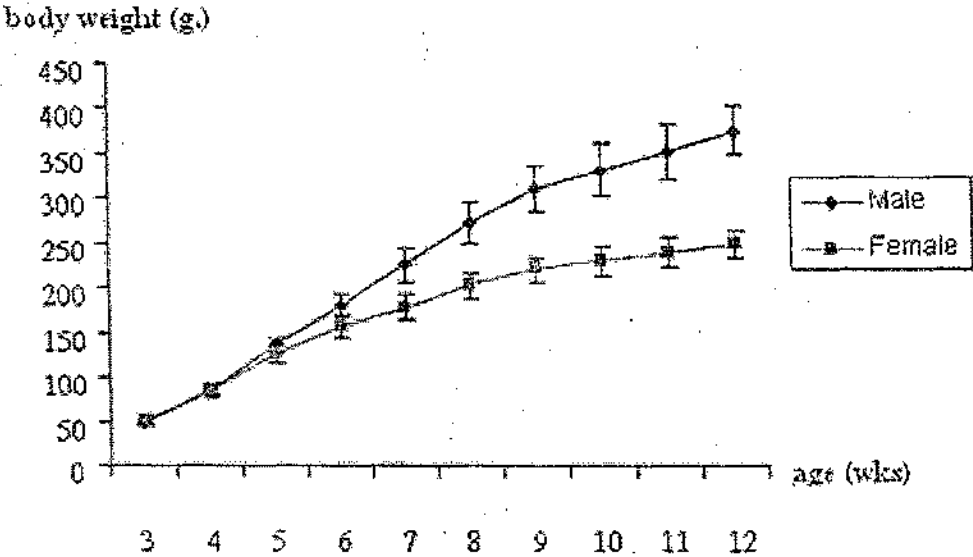


Figure 1: Growth Rates of Male and Female Wistar Rats

In both males and females there is a reduction in the differential lymphocyte count for neutrophils with the difference for females being significant ($p < 0.05$).

p36-37: Mean Clinical Biochemistry Data from Tables 3.01 and 3.02

Test	Sex	Non-Bt Cotton	,Bt Cotton	Significance (t-test)
Total Protein	M	9.38 ±1.5	7.52 ±1.28	ns
SGOT/AST	M	162.24 ±16.47	127.98 ±19.65	$p < 0.02$
SGOT/AST	M	112.14 ± 8.10	137.88 ±2.35	$p < 0.0005$
SGPT/ALT	F	36.88 ±2.69	49.22 ± 6.96	$p > 0.005$
Cholesterol	M	76.2 ± 22.2	51.40 ±12.9	ns

ns - not significantly different

The values for the biochemical indicators are in respective units as given in the original tables. Note the opposite trend in SGOT in males and females for which the differences are significant. SGOT and SGPT are markers of organ integrity. The differences are being pointed out to indicate the casual approach of the toxicologist stating that no differences were observed and the concerned Regulatory process, which seems to be in agreement.

NHH 44 Bt Rat Acute Toxicity dossier: Mean Percentile Organ Weights from p41-42-tables 4.01,4.02

Organ	Sex	Non-Bt Cotton	Bt Cotton	Significance (t-test)
Lungs	M	0.79 ±0.11	1.08 ±0.05	p < 0.001
	F	0.73 ±0.02	0.75 ± 0.02	ns
Heart	M	0.49 ±0.12	0.38 ± 0.05	ns
	F	0.41 ± 0.01	0.33 ±0.03	p < 0.001
Spleen	M	0.38 ± 0.07	0.32 ± 0.05	ns
	F	0.25 ± 0.02	0.38 ±0.01	p < 0.001
Kidneys	M	0.92 ± 0.05	1.00 ± 0.11	ns
	F	0.79 ±0.01	0.85 ±0.06	ns
Uterus	F	0.12 ±0.02	0.23 ±0.09	p < 0.05

At face value several of the figures are significantly different between Bt and non-Bt cotton fed animals, Females show more difference in size of spleen, heart, and uterus.

It is rather rare that a tissue like heart shows reduction in weight and has a similar trend between genders but more pronounced again in the females. The average lung weight differences were highly significant in Bt -Cotton treated males compared to Non Bt- Cotton fed males (Table 4.03, p 43).

In view of the above , the statements in Table 1.03 p22, Summary of Observations - Males and Females stating " No noteworthy findings" with regard to necropsy observations is far from being scientifically valid, as far as the opinion of the toxicologist is concerned . This also raises the question of a major concern, on the role of Expert Committees having examined the dossier -and the Regulatory Body, finally accepting the scientific merits of the study.

3. Metahelix MLS 9124 dossier Goat sub-chronic study:

WBC counts show greater increase from starting values for Bt treated in the case of, both male and female (Tables 5.1 and 5.2).

Examination of the rat 90-day subchronic study reveals the same highly unusual gender parity in growth rates and body weight as was seen in the NHH 44 Bt rat subchronic study discussed above.

p22-23: Tables 1.03 and 1.04

	Mean Body weights (g) Week 0					
	Vehicle	Non-Bt	Bt	Vehicle	Non-Bt	Bt
Males	128. 2	130. 2	126.5 ±4.25	234. 4	233.5 ±4.30	230. 8
Females	131. 9	129. 9	129. 3	236. 6	232.6 ±3.75	231. 9

4. Bt Brinjai dossier:

Toxicology and Allergenicity Studies vol 1, titled Development of Fruit and Shoot Borer Tolerant Brinjai (Mahyco): (i) In the 14-day acute toxicity in rats (p206, p208 - Tables B2.3, B2.4),

Aspartate aminotransferase (AST) levels in both males and females were significantly different between the Group 2 (non-transgenic Brinjai) and Group 4 (transgenic Bt Brinjai) treatments:

Gr 2 males (IU/L): 149.8 + 23.59 and Gr 4 males: 244.8 + 56.4 (p<0.01) Gr 2 females (IU/L): 165.4 ± 16.5 and Gr 4 females: 251.8 ± 51.4 (p<0.01)

AST is a marker of organ integrity and increased AST could indicate damage to liver or heart.

(ii) In the 90-day chronic toxicity study in rats (p272, p274 - Appendix A4; p283, p287 - Appendix B1; p291, p295 - Appendix B2) the following serum, blood, and organ parameters showed significant differences between Group 2 (nontransgenic) and Group 4 (Bt transgenic) treatments for female rats:

Bilirubin: Gr 2 females: 0.595 ± 0.122 and Gr 4 females: 0.81 ± 0.13 ($p < 0.01$)

WBC ($\times 10^3/\text{mm}^3$): Gr 2 females: 9.3 ± 2.67 and Gr 4 females: 13.97 ± 5.49 ($p < 0.05$)

Spleen wt (g): Gr 2 females: 0.81 ± 0.13 and Gr 4 females: 1.02 ± 0.24 ($p < 0.05$)

These significant differences have not been pointed out in the dossier and neither have they been identified by the regulator following examination of the dossier.

The mode of feeding involving suspension of Brinjai powder in vegetable oil and delivery by gavage does not reflect the normal mode of intake and should instead be by incorporation in the feed.

Milk yield in cows:

Lactation study in cows is one component of safety analysis particularly when parts of the GM crop would be used as fodder. In the case of the GFM Cry1A dossier (Nath Seeds), vol 4 (www.igmoris.nic.in) the milk yield study followed a crossover design involving two phases. In the first phase, one set of animals were given Bt cotton seed in their feed for 4 weeks whereas another set were given feed containing non-Bt cotton seed. At the end of that period, the treatment was changed between the two sets of animals so that animals that were fed the Bt material were switched to non-Bt (Bt-nonBt) for 4 weeks and those that had received non-Bt in the first phase were given Bt (nonBt-Bt) in the second phase. The experiment is designed so as to include detection of effects of the treatment after the exposure period. The study concludes that *"milk yield ... did not show any significant difference when compared between both the experimental groups (Table 6 and 7)."* This statement appears to have been accepted by the regulator at face value even though no analysis is presented beyond the calculation of the mean and standard deviation of the daily values for each group. The basis for the conclusion appears to be that the starting and ending values for each group

fall within the range of mean \pm s.d. However, the animals chosen for the study differ widely in the starting values for the milk yield, ranging from 6.0-20.0 kg for one group and 12.5-19.5 kg for another so as to give a starting mean and standard deviation of 14.54 and 3.64 for one group and 16.71 and 2.40 for the other. By including animals with widely differing milk yields within a group, the sensitivity of the experiment is reduced and it is only very large differences that will be detected by this criterion. The ending mean values are 11.8 ± 4.4 and 17.2 ± 3.9 respectively. However if one plots milk yield on a weekly basis for the two groups (Figure 2), a decreasing trend for the Bt-NonBt group is seen, particularly after the changeover point at day 29. Since data is given for each animal, a paired t-test before and at the end of the treatment on a per animal basis can be carried out and when this is done it indicates a significant decrease in milk yield at the end of the experiment ($p = 0.014$) whereas for the NonBt-Bt group, there is no decrease in yield ($p = 0.57$). The mean reduction in milk yield in the Bt-NonBt group is 18.4% with 7 out of 12 animals showing a reduction of 19%-50% at the end of 8 weeks whereas an increase in milk yield is seen in only 1 animal which shows a 15% increase. This is a significant difference which has been ignored because no statistical analysis has been done either by the tester or by the regulator. If analysis had been done and this difference had been noted it would have alerted the regulator to a possible carryover effect of the Bt-cotton seed feed on milk yield and pointed to the need for further studies including for longer duration in order to test the reproducibility of the findings.

In the case of the lactation study for MON531 the range of values in the starting set was also wide, ranging from 4.4-16.1 for Bt (Mean = 7.76; SD = 3.14) and 5.5-11.1 for NonBt (Mean = 8.37; SD = 1.59) in Table 1 of the dossier (vol 4, www.igmoris.nic.in). The Bt and non-Bt samples diverged within the adaptation period itself reflecting that they were not homogenous and represented different populations for the purposes of the experiment. Only mean and S.E. values at the beginning and end of the experiment are

given. Numerical values for each animal for each day have not been provided. The methodology used to calculate statistical parameters and the significance by Student's T test is also not valid as the samples are not random and homogenous. Moreover they have pooled all readings during adaptation (13 days) and experiment (28 days) to give n=130 and 280 days respectively in Table 5 of the dossier whereas n=10 (number of animals) should be used. The number of degrees of freedom (determined by the value of 'n') is an important part of the statistical test and if incorrectly altered can give the wrong results. This point appears to have gone unnoticed by the regulator.

In general the selections should be made after the adaptation period. The requirement for the test should be in terms of being able to detect a certain level of difference as significant instead of a certain number of animals to be used for the test as is currently stated. For studies such as milk yield where parameters are measured before and after the treatment, the data should be presented for each animal on a daily basis and effect of the treatment can be assessed using the paired t-test which does not depend on randomness and normality.

In the case of the JK Seeds Event 1 dossier (vol 6) the lactation study has employed a crossover design and the data reported as average daily milk yield for each week (Table 6 of dossier). Only means have been given and standard deviation has not been indicated. No detailed statistical analysis has been carried out and it has been concluded that there is no difference in daily milk yield as a result of the treatment. However, there are indications in the second phase of the treatment, of a possible declining trend in cows that have been given Bt-cotton feed which would need further examination including additional studies in order to draw any firm conclusions. Had the data been reported in greater detail (daily milk yield per animal), it may have provided further useful and relevant information in this regard.

the case of the lactation study for the CICR NHH44 cotton event, the data has been reported as single point average yields for the adaptation and experimental periods along with standard deviation and it has been concluded that milk production is similar for Bt and non-Bt fed animals. Daily numerical values for each animal have not been provided, however Fig 4 on p20 of the cow feeding study (www.iqmoris.nic.in) shows a graph of daily average milk yield for Bt and non-Bt during the adaptation and experimental periods. During the adaptation period of 19 days, the graphs for (future) Bt and non-Bt sets are largely overlapping and intersect each other several times, however within a few days after the start of the experimental (treatment) period of 28 days, the two lines separate out with the Bt graph showing slightly but consistently lower values than non-Bt. This may indicate the start of a trend of reduction in . yield and would need to be pursued further in a longer study to determine if the difference is real.

For the MLS 9124 study milk yields are reported as mean values and S.D. for 10 animals for the adaptation and experimental period. In addition the mean daily values for Bt and non-Bt are graphically represented (Vol1(10): p20, Fig. 4 of the dossier, www.iqmoris.nic.in) and it is seen that during the adaptation period there is close overlap between Bt and non-Bt, however in the experimental period the two lines separate out, with the Bt graph consistently showing a slightly higher value than non-Bt. This trend is opposite to what is seen for the CICR NHH44 study and would also require further studies to confirm.

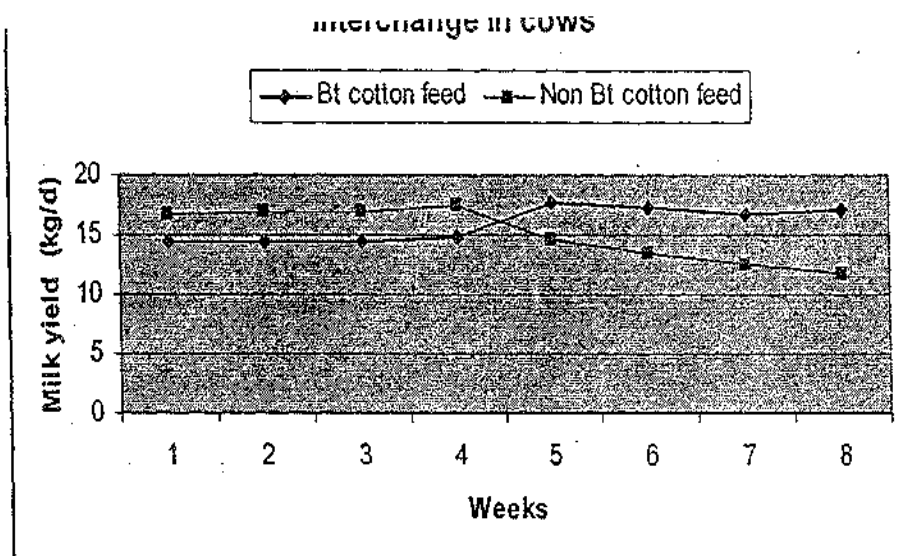


Figure 2: Two groups of cows were first fed on either Bt or Non Bt cotton feed for 4 weeks and later interchanged for the diets with the other cotton feed and daily milk yield is recorded. Milk yield of cows receiving Bt cotton first (line with higher level to start) showed no changes during the first phase and also later. On the other hand the cows receiving Bt feed first (line starting with lower value) showed no changes in the first 4 weeks but showed a decline after switching over to non-Bt feed suggesting a carry over effect of Bt.

Environmental Risk Assessments

Assessing the possible consequences of a GSvIO on the environment is probably the most difficult part of the risk assessment for several reasons. For one, the interactions with the environment can vary widely depending upon the conditions. Secondly, one is dealing with a complex system and testing can involve a high level of uncertainty. Further, many of the effects would not be immediately apparent and may take several years to manifest. An assessment of the potential adverse effects requires knowledge of the relationships and interactions that the GMO may have with other organisms. Often this knowledge is limited or incomplete. This is especially an issue for example when it comes to transgene flow in crops in areas that are centres of Origin or diversity for that species, and for which there are several wild relatives in the environment.

Rather than go into the detailed analysis of environmental tests in individual dossiers, the TEC would like to comment on the nature of the environmental tests that have been carried out and the extent to which they address environmental concerns. The (draft) Guidance for Information/Data Generation and Documentation for Safety Assessment of Regulated Genetically Engineered Plants is articulated in a way which presents different categories of tests to be carried out in checklist fashion. In fact all the tests are grouped as Checklists. This has the effect of simplifying the testing and evaluation process for both the applicant and evaluator. This approach is convenient and may work to some extent for molecular and health safety but is much less applicable for environmental studies where there is greater variability in, the particulars of the tests that need to be done. The CPB and Guidance on Risk Assessment of Living Modified Organisms (UNEP/CBD/BS/COP-MOP/6/13/Add.1) are articulated in terms of issues to be addressed, rather than tests to be carried out, and provide a roadmap for the risk assessment process. This is a more appropriate reference for planning the risk analysis and also meets the requirement with regard to

international obligations.

The report on the Environmental Risk Assessment (ERA) for Bt-Brinjal Event EE-1 by Prof. David Andow gives as its main thesis that "the GEAC set too narrow a scope for the ERA". Elsewhere in the same report it is stated that "Although the GEAC and Mahyco have already invested considerable time and effort into environmental risk assessment (ERA) for EE-1 hybrid Bt-brinjal, much of the effort was misdirected and did not assess actual adverse environmental consequences in India." The report on the Bt-brinjal EE-1 ERA by Dr. Doug Gurian-Sherman comments with regard to gene flow studies that "Given the widespread concern about gene flow, it is remarkable that there appears to be no assessment of possible harm from gene flow from Bt brinjal to wild brinjal relatives in India. ... A few experiments were performed to examine gene flow distances. But this is wholly inadequate, ...". These statements reflect on the underlying situation that ERA is inadequately understood and addressed in the Indian guidelines and regulatory system.

The deficiencies are likely to be a consequence of the way in which ERA has been treated in the guidelines as a set of tests to be carried out instead of issues to be investigated and addressed. This kind of treatment has resulted in oversimplification, omission, and the real purpose of an ERA being missed.

Transgene flow from Bt-brinjal to wild and weedy relatives is a major biosafety concern because of the possibility that it will make the weed more aggressive by reducing its' sensitivity to insect pests, thereby increasing invasiveness. Two recent publications on brinjal (Samuels, 2013a,b) point out that according to the document on the biology of brinjal (<http://dbtbiosafety.nic.in/guidelines/brinjal.pdf>) in the preliminary tests in India in 2007, only four spiny relatives of brinjal were tested for crossability with brinjal and only one (*Solanum incanum* L) was found to be crossable. However, the features of the hybrid progeny were not investigated, in fact over the years, over 50 experimental studies have examined the potential for hybrids to be formed between brinjal and its relatives. The two papers bring out the point that to date it is known that there are six wild relative species and

four cultivated spiny *Solanum* species that occur in India which are known to be able to cross with brinjal to produce reproductively fit hybrids. Transgene flow would very likely occur if these wild relatives are present in the vicinity of Bt-brinjal. There is no evidence so far that increased invasiveness of recipient plants will occur as a result of transfer of Bt, at the same time there is no evidence that it won't occur. The precautionary principle as present in the CPB international guidelines would strongly point towards erring on the side of caution. These papers illustrate the critical importance of having as complete and comprehensive information as possible on the biology of the species when considering release of GMOs. In this case India is considered to be the centre of origin of brinjal and centre of diversity so there would have been all the more reason to be especially watchful. As pointed out (Samuel, 2013a), no GMO intended directly as a food has been commercially introduced into its centre of origin. Yet Bt-brinjal was all set to become the first GM food crop to be used directly as food, for release in India until Ministerial intervention took place.

Summary:

In several cases the reporting of data as well as methods and analysis has been incomplete and cursory. There are also deficiencies in selection of samples, methods of analysis, and statistical tests, making it difficult to draw meaningful conclusions. Nevertheless all the dossiers conclude that there is no significant difference between Bt and non-Bt treatments and this has been accepted at face value by the regulator. In certain cases such as the LOD 0.01% event-specific protocol, a reduced interpretation of the test has been knowingly accepted by the regulator in a form that is no longer event-specific. In at least one case (that of Nath Seeds GFM CryIA), the TEC found evidence of a significant reduction in milk yield following feeding with Bt cotton seed, although the dossier said there was none. There are also indications of possible change in other cases and it would require further evidence and studies to address this conclusively. In the case of GFM CryIA the decline in yield was observed towards the end of the second month after start of feeding. This suggests that one month may be insufficient duration to see an effect (most of the studies involve 4 weeks of treatment). Significant differences between the Bt and non-Bt treatments were also detected in the rat and goat toxicology studies for several events with regard to hematological parameters, serum enzymes, and organ size whereas the dossiers ignore these differences. The number of such cases that have come to the notice of the TEC also reflect on the manner in which the toxicology data has been examined and the Regulatory Body for having accepted the reports.

Based on the examination of the dossiers the following are the overall findings:

1. There are serious deficiencies in reporting of the data in the dossiers and more importantly in the way in which these have been examined and the conclusions accepted by the Regulatory Body. The deficiencies are serious enough that several of the dossiers are unlikely to meet international guidelines. The examination of the dossiers needs to be done with far closer attention to the completeness and quality of the data and to the analysis of the data

with regard to the methodology of the experiments and the statistical tests that need to be employed. In case necessary information for appropriate analysis has not been provided in the dossier, it should be obtained. The regulator has frequently accepted conclusions on health safety in the dossier regarding absence of a difference between Bt and non-Bt studies based on incompletely reported data or without appropriate statistical analysis, to the point of missing a difference where one does exist. Examples of this were found in the lactation studies, and in the blood, biochemistry, and organ parameters and clearly conveys that examination of the data and its analysis by the regulator is deficient.

2. Where significant differences are observed further studies should be carried out to determine if these differences are reproducible and have a basis. Such studies may include repeating of experiments or performing additional tests as determined by the regulator. The regulator may also get such tests performed by one or more independent laboratories.
3. Some tests need to be carried out for longer duration in order to increase the time of exposure so as to detect possible effects with greater confidence. For example, most lactation studies have been of one month duration of giving Bt feed which may be insufficient time to reliably detect differences. It is therefore suggested that the duration of the feeding for lactation studies should be increased to 3 months.
4. There are also issues with regard to the guidelines prepared by the regulator as reflected in the Guidance for Information/Data Generation and Documentation for Safety Assessment of Regulated, Genetically Engineered Plants. The guidance document which draws from international guidelines presents the risk assessment as a set of tests to be carried out whereas the purpose of the risk assessment is to identify potential safety issues and address these through a process of risk assessment as described in the international guidelines. Unless the purpose of the tests is

kept in mind, the risk assessment is likely to fail to meet its

Objectives. This is especially the case with the environmental risk assessment.

Bt Toxins: Mechanism of Action in .Relation to Safety

Bt toxins are members of a family of insecticidal proteins produced by strains of *Bacillus thuringiensis*, a commonly occurring bacterium found in insect-rich habitats and soils. The Bt proteins are made when the bacterium runs out of nutrients, stops multiplying, and forms spores. Bt proteins accumulate as crystalline inclusion bodies consisting of one or more proteins within the , spore. For this reason Bt proteins are also called Cry proteins (for crystal) and different *Bacillus thuringiensis* strains produce different but related sets of Cry proteins. There are over 600 Cry proteins currently known, belonging to three different groups which are distinct from each other ([http://www.lifesci.sussex.ac.uk/home/Neil Crickmore/Bt/toxins2.html](http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/toxins2.html)).

Modes of Use of Bt toxin

Following the recognition of the pathogenic properties of *B. thuringiensis* to an insect pest in 1915, the potential for its use as a biopesticide was recognized and efforts were made to develop methods for its culture and formulation as a microbial insecticide. In 1938 the first commercial formulation of Bt consisting of sporulated cells became available and the mode of action in target species was described in 1956 (Crook and Jarrett, 1991). For many years Bt insecticides made of spore preparations of *B. thuringiensis* var. *kurstaki* (Btk) were used 'only to control lepidopteran (butterflies and moths) insect pests which are specifically infected by Btk, and Btk still forms the basis for many formulations that are currently used. Over the years, screening programmes have also identified other Bt strains which act on different orders of insects. Identification of strains that act on coleopteran and dipteran insects led to the development of control strategies against beetle pest species in agriculture and against dipteran disease vectors (e.g. mosquito species) in public health programmes respectively (Keller and Langenbruch, 1993; Becker and Margalit, 1993). Certain Bt toxins have also been found to be toxic to

nematodes (roundworms)(Bottjer et al., 1985; Wei et al., 2003) which extends the possible uses of Bt toxins against parasitic nematodes. The development and use of Bt transgenic plants (Schuler et al., 1998) where the gene for Bt toxin is incorporated in the plant genome and expressed in plant cells resulting in Bt toxin production *in planta* marked an advance in technology for the use of Bt toxin. Transgenic Bt plants have several advantages over spray formulations. These include: (i) greater duration of exposure as spray formulations are only present on the surface of leaves and aerial portions of the plant for a limited period of time whereas transgenic plants make Bt toxin throughout the life of the plant thereby affording protection throughout the life of the plant; (ii) greater exposure due to presence of Bt toxin in all the cells of the plant especially internal and root tissues where sprays would not reach thereby targeting pathogens that affect inaccessible portions of the plant such as the root; (iii) no treatment of plants is required for delivery and there is no exposure of workers to sprays and potentially allergenic effects of spores in formulations. The widest usage of Bt toxins in transgenic plants has been of the lepidopteran-active CryIA, and Cry2A group although others have also been used. Biopesticide Bt-spray is different in composition from Bt derived from Bt-transgenic plants. Bt transgenic plants exert selection pressure on the insect to develop resistance, whereas Bt-biopesticide does not and wears out in sunlight and rain.

Mechanism of Action of Bt proteins

The biggest and most well studied group of Cry proteins is called the 3-domain group (so named because of the presence of 3 distinct domains in the protein molecule). The 3-domain Cry proteins are produced as inactive precursors within the bacterial spore. When the spore is ingested by the insect larvae, the protein is solubilized in the alkaline environment of the insect gut and gets processed by digestive enzymes present in the gut into a smaller molecule that is the active form of the toxin. The activated toxin can

bind to the invaginated surface of gut epithelial cells (the brush border cells) through interaction with receptor proteins present on the surface of the cell following which it is further processed and able to effect changes in the cell surface ultimately leading to death of the cell (Schnepf et al., 1998).

Investigation of the molecular mechanism of action of Cry proteins have been based to a large extent on the Cry1A toxins (reviewed in Soberon et al., 2009) A large number of studies have examined the mechanism of action of Cry toxins, however the exact mode of action remains to be deciphered and there are different views on how Cry proteins bring about death of the cell . (Soberon et al., 2009). The classical model of how Cry proteins work is that their interaction with receptors present on the cell surface leads to processing and assembly of the Cry protein molecules into oligomeric structures consisting of associated molecules of Cry protein that insert within the cell membrane to form pores, thus causing osmotic shock to the cell which eventually lyses and bursts. The Cry proteins are capable of interacting with at-least two types of receptor proteins on the cell surface as part of the process by which they cause death of the cell. What is considered the primary receptor protein in susceptible insects and is capable of binding with high affinity to Cry toxins is a member of a class of cell adhesion related protein called cadherins. According to the pore formation model, binding with cadherin is considered to facilitate processing of the toxin by cleavage at the amino terminal end of the protein which promotes assembly of the toxin into oligomeric forms. The oligomers have increased binding affinity for secondary receptors attached to the cell membrane which include aminopeptidase N (APN) and alkaline phosphatase (APN) that belong to a class called glycosylphosphatidylinositol (GPI)-anchored proteins (Soberon et al., 2009). Following interaction with secondary receptors, the toxin oligomers

insert into the cell membrane and form pores. An alternative picture of how Cry proteins work is that the binding of Cry toxin to receptors on the cell surface activates a signaling mechanism within the cell that leads to death of the cell (Zhang et al, 2006). It is also possible that there is more than one mechanism of action of Cry protein (as has been observed with other bacterial toxins) and which mechanism operates could depend on the conditions such as the concentration of the toxin as well as as the cell type. For many years it was thought that death of the larvae occurs due to digestive dysfunction caused by damage to the gut, however more recently it was shown that death of the larvae is brought about by septicemia caused by invasion of gut microbes into the larva (Broderick et al., 2006).

How Specific Are Cry Toxins in Their Action?

Cry proteins are a major but not the only insecticidal constituents of the crystalline inclusion bodies present in spores of *B. thuringiensis*. The specificity of Cry protein action has been studied using purified protein preparations made either from bacterial spores or from expression of Cry proteins using a cloned gene in the common bacterium *E. coli*. Historically, most studies to determine specificity of Cry protein action have been based on examining short term acute, lethal effects of feeding the protein on test organisms. Research on chronic, sublethal effects is more limited and recent, arising largely from the need to examine possible direct and indirect effects of Bt transgenics on non-target organisms. Effects of Bt on lepidopteran and other non-target insects have been observed including a direct effect of Bt transgenic pollen on the monarch butterfly (Losey et al., 1999), and effects on the green lacewing (Hiibeck et al., 1998;) and ladybirds (Schmidt et al., 2009;), both of which are predatory insects that are considered to control pathogenic species. The conclusions of the lacewing and ladybird studies have been contested and debated (Romeis et al., 2004; Alvarez-Aifageme et al., 2010; Hiibeck et al., 2012). Other studies have pointed to the presence in streams and possible effects on waterborne insects, of Bt toxins from detritus

left in the field of Bt transgenic crops (Rosi-Marshall et al., 2007; Tank et al., 2010). These studies are part of a growing body of evidence that Bt toxin can have sublethal effects on nontarget insects and other invertebrates. However, more information is required before the actual extent of these effects on non-target species can be assessed.

The ability of Cry toxins to cause lethality in an insect or cell type has been associated with the presence of the cadherin receptor. Expression of the gene for the cadherin receptor Bt-R1 in an insect cell line rendered it susceptible to the toxin (Zhang et al., 2005). Conversely mutations that disrupt the cadherin receptor renders insects resistant to CryIA toxins (Gahan et al., 2001; Morin et al., 2003; Xu et al., 2005). However, certain modified CryIA toxins that lack a small initial portion of the toxin molecule were found to be toxic to insect strains that lacked a cadherin receptor due to a mutation in the gene for the cadherin receptor (Soberon et al., 2005). This result clearly shows that it is possible under certain conditions for the Cry1A protein to kill insects that lack the cadherin receptor. This finding also points to the plausibility of such processed forms of the normal CryIA toxin being generated in the gut even in small amounts which may be sufficient to cause sublethal adverse effects on non-target organisms lacking the cadherin receptor, and which could be significant on chronic exposure. Such potential effects would also need to be considered when evaluating food safety of Bt transgenic crops (see below).

Studies on Cry toxins in relation to vertebrate non-targets are also few although it is generally believed that Cry toxins do not exert an effect on vertebrates as vertebrates lack receptors for Cry proteins. The portion of the insect cadherin that binds CryIAb toxin has been defined (Dorsch et al., 2002) and shows limited relatedness to vertebrate cadherins at the sequence level. However, two studies have provided evidence that Cry proteins can bind to mammalian intestinal epithelial cells. One study carried out on mice found that CryIAC protoxin can bind to intestinal epithelial cells and bring about transient changes in the electrical properties of the intestinal mucosal

tissue (Vazquez-Padron et al., 2000). A second study in cows (Shimada et al., 2006) also found that CryIAb is able to bind to intestinal epithelial cells. No symptoms of acute toxicity were detected, however the possibility of less drastic effects has not been ruled out. A recent study has shown that expression of CryIAc is inhibitory to plant growth and development (Rawat et al., 2011) and this inhibitory effect may be overcome by targeting the CryIAc to chloroplasts. Thus CryIAc also appears to have the capacity to cause unintended detrimental effects to plants.

Emergence of Cry toxin resistance in insect pests

Susceptible insect pests are very efficiently killed by Cry toxins. The high degree of killing of the target insect when it feeds on Bt transgenic plants results in strong selection pressure in favour of genetic variants of the insect that are resistant to the Cry toxin. These variants are preexisting in the insect population but make up a very small proportion. However under conditions where they enjoy a large survival advantage, their number can increase and assume a major fraction of the population resulting in the erosion of insect resistance in the Bt transgenic plant. The known naturally occurring genetic mechanisms for Bt toxin resistance in insects are recessive (both gene copies in an individual need to be the resistant ones for the insect to be resistant to the toxin). Based on the recessive nature of the resistance mechanisms, one strategy that has been recommended is the use of refugia where a small amount of the sensitive crop or alternate host for the insect is grown alongside the Bt transgenic. This strategy is designed to maintain a certain population of the (sensitive) insect and increase the likelihood that the resistant gene copy will always be present in an individual insect alongside a sensitive gene copy so that resistance is not expressed and the insect population remains sensitive. The use of refugia has been widely deployed for Bt crops in different countries and the results suggest that refuges have helped to delay the emergence of resistance (Tabashnik et al., 2008). One issue with the refuge strategy is that of compliance. In the USA, compliance rates for Bt corn have declined from 90% in 2003-2005 to 75% in 2008 (CSPI Report, 2009). In India GEAC has stipulated a refuge area of 20% for Bt cotton, however, the compliance is likely to be far

weaker as has been recognized in the Mayee Committee Report (2006) due to small land holding and economic pressure to maximize gains as well as limited ability to enforce regulatory measures at the field level. Refugia as a general strategy to delay emergence of resistance are therefore unlikely to work in the Indian context. Alternatives such as mixing of resistant seeds with sensitive ones have been suggested, however the efficacy is untested in practice and it is likely that this will not be favoured by the farmers.

The emergence of resistance to CryIAc in the pink bollworm cotton pest has been reported in Gujarat (Bagla, 2010) and in China (Wan et al., 2012). In Gujarat significant levels of resistance in pink bollworm were noticed in 2009, seven years from the start of adoption of Bt cotton. In the case of the American bollworm, the CryIAc gene present in Bt cotton is still effective in providing protection, however the concentration of CryIAc toxin required to kill the field strains of the American bollworm has steadily increased since the start of adoption of Bt cotton in 2002 (Kranthi, 2012), so it is likely that protection will break down at some point. One way to delay resistance is to use two Cry genes that have differences in their mode of action. The likelihood that a resistance mechanism will operate against both genes is therefore expected to be much lower than against a single gene. While this is generally regarded to be the case, recent results have shown that this need not always happen. In this case selection for resistance against Cry2Ab was found to also cause resistance against CryIAc (Tabashnik et al., 2009).

Health and food safety of Bt transgenics

For most foods safety has generally been established based on a history of safe use* rather than testing for safety. The considerations for testing human health and food safety of transgenic plants including Bt transgenics have been modeled on drug testing and involve acute exposure studies as well as subchronic exposure studies in animals. These studies involve the use of a small number of animals (typically 5-10) which are given doses of the drug

that can be very high and one assumption of the test is that adverse effects if present would be manifested by all or most of the animals being tested. This assumption may not hold for foods. A second difference is the length of exposure. Whereas most drugs would be given for a limited period of time, food would be consumed throughout the life of an individual, hence the duration of exposure is much longer in the case of food than for drugs. A third difference is the number of people who would be exposed and the ability to track these 'people: for drugs, the number of people taking a drug is a small fraction of the total population and they can be monitored whereas in the case of food, very large sections of the society, potentially the entire population would be exposed. Even if a very small percentage of consumers suffer adverse effects, the total number of people affected can be very large. Hence the level of acceptable risk is very low for transgenic foods and a very high level of certainty of absence of adverse effects would be required.

In the case of Bt which is a toxin (albeit against insects), one needs to be very sure that there are no adverse effects in humans. There are still a number of gaps in our knowledge of the mechanism of Bt toxin action. There is also evidence that the toxin may operate through more than one mechanism depending upon the conditions. CryIA toxins have also been seen to bind to the mucosal membrane of the vertebrate intestine. It is therefore critically important that potential adverse physiological consequences of any kind, not just short term lethal effects be ruled out with a high degree of confidence. In the view of the TEC this would require a substantial amount of additional studies that include chronic and intergenerational feeding studies so as to rule out possible longterm unintended effects. The TEC is aware that in countries such as the USA, Bt corn is being grown and used mainly as animal feed and in processed food for human consumption for a number of years. However, there is no specific information as to how much transgenic corn is directly used as food for human consumption and how much is used for oil or processed food and fodder. It is likely that most of the transgenic corn is used for fodder and processed food

and the actual amount that is directly being used for human consumption is small and does not represent a major part of the diet. Because there is no . labeling of transgenic food in the USA, this information is not readily available. However in India, several of the food crops for which Bt transgenics are being made would be mainly used directly for human consumption. The TEC is of the view that the only way to address possible longterm unintended effects of Bt is to include chronic and transgenerational toxicity studies in the set of tests to be carried out. It would take a substantial amount of time as well as analysis for a number of such studies to be carried out on a number of events worldwide and the results analyzed before the general safety of Bt in food crops can be established, in addition there need, to be specific studies on each event intended for commercialization to examine the possibility of unintended longterm effects. The present status of information on safety of Bt transgenics does not justify commercialization of Bt food crops and it would be an unnecessarily risky proposition to go ahead with this. This was the basis for the recommendation of a moratorium on Bt transgenics in food crops in the interim report of the TEC.

Herbicide Tolerance (HT)

Introduction

Traditional methods for control of weeds in agriculture have relied to a large extent on cultural and mechanical practices such as tilling of the soil and manual removal of weeds. The identification of chemicals that cause inhibition at low concentration to growth of plant species led to the development of synthetic herbicides. The first synthetic herbicide was 2,4-dichlorophenoxyacetic acid (2,4-D) developed during WWII research on plant physiology. Since that time a large number of synthetic herbicides have been developed. Some of these herbicides are synthetic analogues of endogenous plant growth regulators. Others act as inhibitors of enzymes that participate in essential plant physiological processes. Herbicides can be classified according to different criteria: broad spectrum herbicides act on a large number of plant species whereas selective herbicides act on a few plants; pre-emergence herbicides act prior to or during germination and post-emergence herbicides act at later stages once the plant has become

established; systemic herbicides are transported throughout the plant in contrast to contact herbicides whose action is localized. Herbicides have been applied to crops either singly or in combination. Conventional herbicide treatment is part of a collection of practices that is employed to reduce weeds and pests (Integrated Weed/Pest Management) and often combinations of herbicides are used to control weeds so as to increase effectiveness. These practices include rotating crops and herbicide treatments to reduce the adverse effects of any one chemical on the soil, environment, and ecology, by allowing a period of recovery following application of the herbicide. Use of herbicides in agricultural practice started to gain ground in the 1950s and 1960s when several new classes of herbicides were also identified.

The health safety of herbicides has been considered according to their acute toxicity as well as effects with regard to specific health concerns such as carcinogenicity, reproductive and developmental toxicity, and endocrine disruption. Both WHO and the U.S. EPA maintain lists of chemicals including herbicides classified according to their acute toxicity. When considering toxicity it should be kept in mind that it is possible for a chemical to not show adverse effects in short term acute studies but do so on long term exposure even at low concentrations. Information relating to long term chronic toxicity is limited for most herbicides.

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Classes of herbicides based on usage and mode of action:

Foliar Applied Herbicides:

Downwardly mobile herbicides:

Growth Regulators - These include 2,4-D, dicamba, and picolinic acids (picloram, clopyralid, tricyclopyr). These compounds are analogues of the plant hormone auxin and disrupt the normal growth and development of the plant.

Aromatic amino acid biosynthesis inhibitors - These include glyphosate, and sulfosate. Both are non-selective herbicides.

Branched-chain amino acid biosynthesis inhibitors - Examples of this category are sulfonyl urea herbicides such as chlorsulfuron, sulfoanilides such as flumetsulam, and imidazolinones such as imazapyr.

Chlorophyll/Carotenoid pigment inhibitors - These include norflurazon and fluridone and block the synthesis of green and yellow pigments.

Lipid biosynthesis inhibitors - Examples of these are the aryloxypropionates {fenoxaprop, fluazifop-P) and the cyclohexanediones (clethodim, sethoxydim). These are used to remove grass species and cause discolouration and disintegration of the apical growing portions of the plant.

Upwardly mobile herbicides:

Photosynthetic inhibitors - These include the triazines (atrazine, simazine, prometon, hexazinone), the uracils (bromacil, terbacil), and the phenylureas (linuron, diuron, terbuthiuron)

Contact herbicides:

Cell membrane disruptors – these include the bipyridiliums (paraquat, diquat), the diphenyl ethers (acifluorfen, lactofen, oxyfluorfen)

Glufosinate - Acts to inhibit glutamine synthetase, it is a rapid acting, non-selective, post-emergence herbicide and leads to cell membrane disruption and cell death. Glufosinate shows limited mobility in the plant.

Herbicides applied in soil:

These are mostly pre-emergence herbicides and include compounds that inhibit cell division in the shoot (e.g. thiocarbamates - butylate, cycloate, EPTC), root (dinitroanilines - oryzalin, trifluralin, ethafluralin), or both (dithiopyr, bensulide, napropamide).

Herbicide tolerance crops

The finding in the 1970s that weeds can become resistant to herbicides led to an interest in the development of crops that were herbicide resistant. The advantage of herbicide resistant crops is that they allow more intensive use of the herbicide on the crop thereby simplifying herbicide treatment to use of a single herbicide while reducing the need for tilling of the soil. Herbicide resistance crops can be made either by mutation followed by selection of a herbicide resistant variant, or else by introduction of a transgene that confers resistance to the herbicide. Herbicide resistant crops have been grown commercially since 1984 when oilseed-rape that was triazine resistant was developed and commercially grown in Canada. This was developed by conventional breeding wherein triazine resistance was crossed in from a strain of rape that was resistant, into the commercial variety (Hall et al., 1996). HT crops are presently grown in about 80% of the total area deployed for GM crops worldwide. Soybean accounts for the major share of HT crops and the most widely used HT trait is glyphosate resistance. Glyphosate was found to be a herbicide in 1970 by J.E. Franz working in Monsanto, and was introduced into the market as such in the early 1970s. Glyphosate has been claimed as an ideal herbicide (Duke and Powles, 2008) based on its broad

spectrum activity, low toxicity (LD50 - 50% lethal dose, greater than 5000 mg/kg), and low mobility in soil. Soil studies have found glyphosate half-lives (time for a 50% reduction in amount) ranging from 3 to 130 days (U.S. EPA, 1990; USDA, 1984) so its stability is likely to depend on the conditions. Glyphosate in the soil binds minerals and is also degraded to glyoxylate and AMPA (aminomethylphosphonic acid). Glyphosate was used from 1974 to 1996 as a conventional herbicide. With the advent of glyphosate tolerant crops starting in 1996, usage of glyphosate increased dramatically, displacing other herbicides, and glyphosate has now become one of the most widely used agrochemicals in agriculture occupying about 25% of the global herbicide market. Worldwide about 650,000 tonnes of glyphosate products were used in 2011 (ISAM (2012) Global Status of Commercialized Biotech/GM Crops: 2011 ISAM Brief 43-2011).

A concern that has been expressed about glyphosate relates to its property of being a chelator of metal ions. Overuse of glyphosate has been considered to interfere with mineral nutrition of the plant and lead to reduction in nutritional quality and increased disease susceptibility (e.g. Johal and Huber, 2009). This view has been contested (Duke et al., 2012) and the issue is under debate.

Emergence of herbicide resistant weeds

For some herbicides such as atrazine and acetolactate synthase (ALS) inhibitors, there were several pre-existing cases of resistant weeds present in the environment and these started to emerge soon after the deployment of these herbicides. This was not the case for glyphosate and there were publications at the time that glyphosate resistant crops were first deployed that glyphosate resistance was unlikely to evolve (Padgett et al., 1995; Bradshaw et al., 1997). In the 22 years (1974-1996) that glyphosate was used as a conventional herbicide, weeds that had evolved to become resistant to glyphosate were very limited (Powles, 2008).. However, after the advent and extensive deployment of glyphosate resistant crops (soybean, cotton, corn,

and canola), evolved resistance to glyphosate started to appear. Weeds resistant to glyphosate began to emerge as a significant problem in 2000 and by 2005 had led to an increase in the use of herbicide for soybean and cotton (Benbrook, 2012). The extremely widespread adoption of glyphosate resistant crops has been considered to have created a number of conditions that have combined together to result in the emergence of glyphosate resistant weeds. These are: (i) the strong and persistent selection pressure for glyphosate resistance arising from repeated and continuous application of glyphosate often in successive cycles of crop growth as for example in corn/soybean rotation in the US Midwest where these two crops are often in continuous rotation on the same field. This results in glyphosate application year after year on the same field creating conditions for the evolution of glyphosate resistance in weed species, (ii) The reduction in herbicide diversity arising from the replacement of other herbicides that were previously used, by glyphosate resulting in a single type of selection pressure; and (iii) adoption of no-tillage agriculture, relying exclusively on chemical application of glyphosate to kill all weeds, further contributing to a reduction in the diversity of methods for weed control (Duke and Powies, 2009).

A recent analysis of herbicide usage in the U.S. based on publicly available USDA data has concluded that over-reliance on a single herbicide (primarily glyphosate) has resulted in the emergence of herbicide resistant weeds that have spread to an estimated 20-25 million hectares and caused an estimated increase of 239 million kilograms of herbicide between 1996 and 2011 amounting to ~0.27 kg/ha over what herbicide use would likely have been in the absence of HT cultivars, and resulted in reduced effectiveness of the herbicide (Benbrook, 2012). The emergence of glyphosate resistant weeds provides a striking example of the consequences that can result from excessive reliance on a single herbicide. The problem of glyphosate resistance weeds in the USA is considered very serious. One report likened the magnitude of the problem of weed resistance to glyphosate and other herbicides as "a tsunami still out to sea but approaching land" (Marker et

ah, 2012).

The overall consensus among weed management experts is that there needs to be a reduction in the planting area of glyphosate resistant HT seeds in the U.S. in order to keep weed resistance under control and that sustainable control of weeds will require the application of integrated weed management strategies rather than relying on single herbicides (Mortensen et al., 2012; Duke, 2011; Harker et al., 2012). However, in response to the outbreak of glyphosate resistant weeds, the agribiotech industries are developing crops that will have combined resistance (stacking) to glyphosate and other herbicides such as synthetic auxin herbicides. There are several concerns with stacked herbicide tolerant strategies. Firstly, crops with stacked herbicide resistance are considered likely to increase the severity of resistant weeds, given that cross-resistance to more than one herbicide is known to occur in a number of cases (Valverde, 2003), an extreme case being a biotype of rigid ryegrass which exhibited resistance to nine herbicide classes representing five modes of action categories (Burnet et al., 1994). Secondly, these crops would encourage increased use of the herbicides with likely negative consequences for biodiversity in agricultural fields and the environment. The "short-term fix" that stacked herbicide resistance would provide (Mortensen et al., 2012) would work for a few years but would be unlikely to provide a sustainable solution that is particularly important and needed for countries such as India which have very limited scope for increasing agricultural land area. There is a need to place greater reliance on integrated weed management rather than relying *on* one or two herbicides which adoption of HT would promote.

A recent study compared the overall performance in terms of yield of the US agrosystem that has incorporated GM technologies (mainly herbicide tolerance) with countries of Western Europe which are comparable in terms of latitude, climate, socioeconomic conditions, and food security but have not adopted GM technologies (Heinemann et al., 2013). It found that average yields per unit area in maize from 1961 to 1985 years were higher for USA, but from 1986 to 2010 (GM maize was first grown in the US in 1997) W Europe has equaled or slightly exceeded USA. Yields of maize in the USA

also show greater variation from year to year than in W. Europe. In the case of rapeseed, yield advantage in W Europe since 1986 has exceeded that of Canada which started to grow GM canola in 1995. Furthermore herbicide use has declined to around 80% relative to the usage in 1995 whereas in USA it has increased to 108%. in the case of insecticide usage, the levels in USA have come down to 85% of pre-GM levels, however in W Europe (e.g. France) the levels are down Jo less than 20% that in 1995 even without the use of Bt-transgenics. The authors infer that Western Europe has adopted a set of biotechnologies that equal or surpass that of North American biotechnologies (i.e. HT and Bt) in performance, stability, and sustainabiility. They go on to state that when viewed in global aggregate terms, the performance of GM technologies that have been applied so far does not provide compelling evidence of the benefits that are supposed to accrue over that of other technologies and practices, and a weakness of the current practice of GM is that it is part of a commercialization strategy that aims at greater uniformity (both of crops as weir as herbicide/pesticide treatment) that increases vulnerability by reducing diversity of cropping systems.

Effect of HT on Biodiversity and Environment

Experimental studies of the possible impact that HT crops can have on the biodiversity and abundance of wildlife have been limited. Arguably the largest study was carried out in the UK over a period of four years between 1999 and 2003 and examined 273 trial fields across different locations that comprised maize, spring rape, and beet all of which were herbicide tolerant (The Farm Scale Evaluation of spring-sown genetically modified crops'). The researchers, monitored insects and plants along the edges of the fields. The results indicated that there were differences in the abundance of weeds and insects between HT and conventional herbicide treatments and these differences were consistent across different locations and from year to year. The greater the number of weeds, the more insects there were as weeds provide a foraging ground for insects. The differences also depended upon

how farmers manage the conventional and HT crops and the weed control treatment used for the conventional crops. In India where manual weed control is a significant component, its replacement by application of high amounts of a broad-spectrum herbicide may negatively impact biodiversity in farmlands.

HT in the Indian context

There appears to be a fundamental incompatibility between greatly increased intensity as well as persistence of usage of one or two herbicides as a feature of HT technology, and sustainability of weed management in the Indian context. Comparison between USA where evolved glyphosate resistant weeds have arisen, and Canada where they have not, shows that on average HT canola is grown on a particular cropping field in only one year in four in Canada (Duke and Powies, 2009), whereas in the USA, glyphosate has been successively applied for several years together. The deployment of glyphosate in Canada has therefore been under conditions of far lower selection pressure for emergence of glyphosate resistance than in USA. In the case of India, the availability of land is very much less than USA and Canada, so there is much less room for control of deployment. In fact if HT traits (single or stacked) are applied in Indian agriculture, then one can expect that compliance to recommendations for sustainability such as rotation of crops and herbicide treatments is likely to be low as there is already a tendency among Indian farmers, especially small-scale farmers to overapply pesticides (e.g. Andow, 2010: Bt-brinjal, Finding 6, p13). Use of herbicides on HT crops in India is likely to be driven more by considerations such as performance of the herbicide, convenience, and differences in economics of different HT treatments even if these are small, rather than compliance to conditions for reducing selection pressure for evolution of resistant weeds. All of these would contribute to lowering the sustainability of HT crops.

Another consideration in the Indian context is a socioeconomic one wherein a significant part of the large agricultural workforce, particularly landless labourers is employed for manual labour in the fields including tilling and weeding operations. The small average size of agricultural land holdings

makes this realistic whereas HT traits have greater impact in large scale agriculture systems where tilling and manual weeding require greater investment and there is limited availability of manual labour. Introduction of HT crops would be likely to reduce access to employment for some of the vulnerable sections of rural society. While it is true that in some areas of India there is shortage of agricultural labour and the introduction of HT crops may benefit this section of farmers (at least in the short term), the overall impact that HT crops would have in terms of reducing jobs for agricultural labour in the fields is likely to be more significant.

Conclusions

The major concern with HT approaches is the excessive reliance on increased amounts of one or two herbicides which results in strong selective pressure for the emergence of herbicide resistant weeds and a negative impact on sustainability. The benefits of HT crops in the Indian context may be short term and variable. In addition they are likely to be accompanied by negative socioeconomic consequences as well as on the environment. There are alternatives to HT and it is likely that integrated weed management approaches (not stacking) as part of a basket of technologies would be more appropriate in the Indian context.

Recommendations

Based on the deliberations of the TEC and particularly the examination/study of the safety dossiers, it is apparent that there are major gaps in the regulatory system. These need to be addressed before issues related to tests can be meaningfully considered. Till such time it would not be advisable to conduct more field trials:

1. A secretariat comprising dedicated scientists with area expertise as well as expertise in biosafety needs to be established. This will require consultation with experts having experience at the international level in biosafety testing and evaluation of GM safety dossiers in reputed regulatory bodies. The TEC recommends doing it in collaboration with the Norwegian Government and GM regulatory body since the Norwegian system has an established commitment and experience in is one of the few that are attuned

to considering socioeconomic issues that would be important in the Indian context. The regulatory body should have area-wise subcommittees/expert groups in for example:

- | Health (human and animal)
- | Environment and Ecology
- | Agroecconomics and Socioeconomics
- | Molecular biology
- | Entomology
- | Agricultural and Aquaculturai Systems
- | Public Health
- | Soil science and microbiology
- | Plant biology
- | Regulatory toxicology
- | Plant and animal breeding and genetics

A single committee such as the GEAC or RCGM doing all the evaluation is not sufficient.

2. Conflict of interest in terms of location of the regulatory body needs to be addressed. The suggestion of the TEC is that the regulatory bodies be located in the MoEF. (environmental safety) and the MoHFW (health safety). At a different level, it is self-evident that members of the regulatory bodies should also be free of conflict of interest.

3. Specific sites for conducting confined field trials need to be designated, certified, and sufficient mechanisms put in place- for monitoring the trials and ensuring restricted access, disposal of material, associated testing and other facilities. These sites should be used only for field trials of GM crops (GM and

control material). The sites could be in ICAR institutes or State Agricultural Universities and required conditions for isolation should be established and supported appropriately by ICAR. Sites in company premises may also be considered for certification for trials, however the land should be permanently owned by the applicant/tester. Trials should not be conducted on leased land so as to avoid the possibility that it may be used for a different purpose following the trials.

4. Stakeholder participation, need, socioeconomic considerations, societal impact, and sustainability should be some of the dimensions to be incorporated in the risk assessment and this should be done at an early stage in the risk assessment process.

Tor A: Nature and sequencing of risk assessment and point of release for Open Field Trials

1. It is recommended to introduce a consultation step to start with, ideally prior to the GM product intended for field trials having been developed, wherein the applicant provides information to the regulator about the product, its purpose (including whether it is intended for research only or commercialization), and how it is to be deployed in the Indian context. At this stage the scope of issues that need to be addressed relating to health and environmental safety can be discussed and defined on a casewise basis keeping in mind the overall phases of risk assessment: hazard identification; hazard characterization; exposure assessment; risk characterization; and mitigation options. Need, socioeconomic factors, and sustainability should also be considered and thoroughly discussed at this stage with involvement of all the stakeholders. If a GMO is initially declared for research and at a later stage it is desired to consider it for commercialization then that would be treated as a fresh application. The overall process of risk assessment should follow the Flowchart for the Risk Assessment Process in the Guidance on Risk Assessment of Living Modified Organisms (UNEP/CBD/BS/COP-MOP/6/13/Add.1) of the Cartagena Protocol on Biosafety, particularly keeping in

mind the need to bring out the uncertainties in the assessment. In the case of health safety, the regulator should expect a suitable response to all relevant paragraphs of the Codex Alimentarius Commission (CAC) Guideline for the Conduct of Food Safety Assessment of Foods Derived From Recombinant-DNA Plants (CAC/GL 45-2003) and any other chosen risk assessment procedure, in so doing the regulator establishes a minimum expectation of the risk assessment meeting international requirements. The completion of the risk assessment It needs to be pointed out that both the CPB and CAC guidelines provide guidance with regard to principles and issues that are to be addressed. They leave open the details of specific tests to be carried out which is left to the national system and the regulator.

2. There is a need to include chronic and transgenerational toxicity testing in feeding studies of rodents based on the fact that food is consumed over the entire lifetime and that nutritional stress can also lead to adverse or unintended effects over longterm exposure. The sensitive stages of reproduction also need to be included.
3. The regulatory process should be open to new scientific information that may have a bearing on the risk assessment, if necessary even after deregulation of an event.
4. The applicant is responsible for providing to the regulator, all information that has a bearing on the risk assessment, regardless of whether it was obtained for the purpose of the risk assessment. In cases where the applicant is a collaborator/partner/subsidiary in the development of the GMO, the applicant should provide this information along with the consent of all parties to provide such information.
5. The single largest number of applications for field trials to GEAC are for Bt transgenics including in food crops such as rice. With regard to the nature of tests for Bt in food crops, the TEC was of the view that the safety of Bt transgenics with regard to chronic toxicity has not been established and this needs to be done before it can be considered safe. In this regard it may be

noted that by far the largest deployment of transgenics worldwide is in soybean, corn, cotton, and canola, all of which are used primarily for oil or feed after processing. Nowhere are Bt-transgenics being widely consumed in large amounts for any major food crop that is directly used for human consumption. The TEC could not find any compelling reason for India to be the first to do so. The TEC therefore reiterates its recommendation made in the Interim Report that there should be a moratorium on field trials for Bt in food crops (those that are directly used for food) intended for commercialization (not research) until there is more definitive information from sufficient number of studies as to the longterm safety of Bt in food crops.

6. The second largest number of applications were for HT crops. The TEC has examined the issues in relation to HT, particularly with regard to sustainability and the likely socioeconomic impact on major sections of rural society. On both these counts, based on the reasons presented in the section on Herbicide Tolerance, the conclusion of the TEC is that HT crops would most likely exert a highly adverse impact over time on sustainable agriculture, rural livelihoods, and environment. The TEC finds them completely unsuitable in the Indian context.

7. The first GM food crop to be approved for release in India was Bt-brinjal, in 2009. The recommendation was not accepted by the then Honourable Minister of Environment and Forests Mr. Jairam Ramesh who placed a moratorium on the release of Bt-brinjal event EE-1. The centre of origin of brinjal is believed to be India (Samuels, 2013a) and India is also a major centre of diversity of brinjal with approximately 30 species of wild and cultivated relatives being found in India (Samuels, 2013a). These include potential weed species which can cross to brinjal: *Solanum insanum* a weedy brinjal is well known to form fertile hybrids with cultivated brinjal (All and Fujieda, 1989); fertile hybrids of *Solanum incanum* and brinjal are also known to occur (Deb, 1989); *Solanum cumingii* has been reported as a weed of rice

(Lubigan, R.T. and Moody, K. (1987). IRRN 12:1, p24) and is known to be able to cross with brinjal and form reproductively fit hybrids (Samuels, 2013a). Overall at least six wild relatives and four cultivated species are known to form fertile hybrids with brinjal. The risk of an insect resistance gene being passed on to a weed and increasing its weediness is therefore very real. There are also weedy rice relatives of cultivated rice which can form fertile hybrids with cultivated rice (e.g. *Oryza sativa* f. *spontanea*) where the same concerns arising from flow of transgenes are present and there are several applications for field trials before GEAC for Bt-rice as well. It should be noted that has been advised when it comes to release of GM crops into their centres of origin, i.e. the geographical regions where the crops were domesticated and where wild relatives and weedy forms persist (Papa and Gepts, 2004). To date, no GMO that is intended primarily and directly for food production has been commercially released into its centre of origin (Samuels, 2013a). In the US, there are restrictions on the growth of Bt-cotton in Hawaii (note that cotton is not even a food crop) where *Gossypium tomentosum*, a weed related to cotton is found (Manjunath , 2011: Safety of Bt-cotton: Facts ailyay Fear).

Crops in their centres of origin and diversity often have a deep cultural significance that can easily get lost when utilitarian issues predominate the discourse. Ceremonial and medicinal varieties can also be put at risk from GM crops by reduction of diversity and genetic purity. For example in the case of brinjal, the Malapur variety in Kamataka is an essential accompaniment at temple festivals and religious ceremonies. Likewise, *Oryza nivara* a medicinal rice can also be at risk if GM rice comes to dominate the crop as has happened for example in cotton in India.

The release of a GM crop into its area of origin or diversity has far greater ramifications and potential for negative impact than for other species. To justify this, there needs to be extraordinarily compelling reasons and only when other choices are not available. GM crops that offer incremental advantages or solutions to specific and limited problems are not sufficient reasons to justify such release. The TEC did not find any such compelling

reasons under the present conditions. The fact is that unlike the situation in 1960s there is no desperate shortage of food and in fact India is in a reasonably secure position. The TEC therefore recommends that release of GM crops for which India is a centre of origin or diversity should not be allowed.

Tor B:

The sequence of testing should be carried out in order of increasing environmental exposure required to perform the test. Tests should be done under the minimum conditions of exposure required for the test. The testing therefore proceeds in a progressive manner that increases confidence regarding safety, with increasing exposure. Since risk assessment is carried out on a casewise basis, and the specifics of the risks and tests can vary, it would be beyond the scope of this report to attempt to cover all tests for all crops. Nevertheless there are certain minimum tests which it should be possible to carry out under contained conditions within the laboratory or greenhouse, before the GMO is first taken out of containment. These include tests based on bioinformatics such as possible allergenicity and toxicity; acute toxicity of the purified protein; in-vitro digestibility and any other biochemical tests on the purified protein. In many cases the consideration of first point of emergence from contained conditions arises at the time of event selection. Where appropriate and necessary, tests such as for general growth characteristics and plant habit as part of event selections may be performed under confined conditions in consultation with the regulator. Those tests on the plant that can be performed under contained conditions as judged by the regulator on a casewise basis, should be performed under contained conditions.

To'rC:

Since the GMO is likely to be ultimately intended for growth under open field conditions, the issue is whether it is possible to do an evaluation under

contained conditions. There is published evidence that the characteristics of a GMO can differ significantly depending upon whether it is grown in the greenhouse or in the field (e.g. Zeller et al., 2010). Therefore it cannot be said that it is generally possible to replicate the conditions for testing under different agro-ecological regions and conditions in the greenhouse.

Tor D:

Specific sites for conducting field trials need to be designated, certified, and sufficient mechanisms put in place for monitoring the trials and ensuring restricted access, disposal of material, associated testing and other facilities. These sites should be used only for field trials of GM crops (GM and control material). The sites could be in ICAR institutes or State Agricultural Universities and required conditions for isolation should be established and supported appropriately by ICAR. Sites in company premises may also be considered for certification for trials, however the land should be permanently owned by the applicant/tester. Trials should not be conducted on leased land so as to avoid the possibility that it may be used for a different purpose following the trials.

Tore E:

Tests for detecting contamination at the stipulated level (0.01%) are possible and have been demonstrated in some of the dossiers. However it should be emphasized that these in themselves do not preclude material from escaping. There are several ways in which contamination can occur and it probably will not be possible to deploy the tests at a level that will preclude the possibility of escape. Even in the most careful of conditions contamination can occur. There are well-known examples of contamination having occurred in the US such as that of Liberty Link 601 rice in 2006 for which the commercial damages in terms of export losses led to a settlement amounting to \$750 million dollars (<http://www.digitaljournal.com/pr/419329>). There is also an ongoing case of an unauthorized glyphosate resistant wheat contamination which was recently detected in Oregon, USA. It may also be appropriate to point out that contamination and potential damage can occur even after deregulation especially in the context of an environment in which there is labeling which there is in India (in the USA there is no labeling so mixing of

deregulated GM with non-GM is not an issue). The Department of Agriculture informed the TEC that in India it will not be possible to separate GM from non-GM given the existing systems (the same position has been expressed in the PSC report) and this is probably an accurate assessment. There are also trade implications for example for food export as in the case of rice which amounts to Rs. 12,000 crores annually for India. There is currently no international consensus on the issue of labeling of GM and non-GM which can affect trade in various ways, mostly with GM. having a negative impact on trade because of consumer apprehension and as reflected in government policies in different countries.

TorF:

It is very difficult and beyond the scope of this report to comment comprehensively on the various institutions and laboratories in India with regard to facilities and professional expertise. This would require detailed review of the institutions themselves. In all likelihood many and perhaps most of the institutes/laboratories may not be short of the facilities. However, based on the review of the dossiers the professional expertise and standards across the institutions appears unsatisfactory. It should also be mentioned that it is ultimately the expertise available in the regulatory system that sets the standards for conducting and evaluating the biosafety tests. Unless this expertise and capacity is present, no amount of facility creation will be able to address the issues. Based on the examination of the safety dossiers the TEC has found in unambiguous terms that at present, the regulatory system has major gaps and these will require rethinking, investment, and relearning to fix. A deeper understanding of the process of Risk Assessment is needed within the regulatory system for it to meet the needs of a proper biosafety evaluation. This is not available in the country at present. It is therefore recommended that the requisite understanding be developed through consultation, collaboration, and capacity building. One such successful collaboration has been between Norway and South Africa. Norway has a very comprehensive and reputed biosafety regulatory system and capacity building is one of their mandates. Norway is also one of the few countries that includes socioeconomic impact (especially on developing countries) as one of the dimensions of evaluation. This would be a key factor for India and it is of critical importance that the Indian regulatory system develop the ability to

assess how any GM product is likely to impact different sections of society. The TEC therefore recommends that India establishes a collaboration with Norway for developing a strong and state of the art biosafety regulatory system. This cannot be done in a hurry and will require time. However much time it may require, unless the conditions are met, the required standards of biosafety tests will not be upto the" mark. The process of conducting risk assessments is difficult, and addressing the issues taking into account the various uncertainties requires fulltime attention, and a sophisticated level of area expertise together with training in biosafety. It is the view of the TEC that there is a need to recognize our limitations and work towards developing a regulatory system in consultation and collaboration with experts from a reputed regulatory system such as the Norwegian one in tune with the national and societal needs.

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