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Data Requirement for Safety Assessment of GE Plants in India

- **Protocols for Food and Feed Safety Assessment of GE crops**

- **Department of Biotechnology**
- **Ministry of Science and Technology Government of India**

- **2008**

Guidelines were based on international best practices

- Codex Alimentarius Commission (CAC)
- Food and Agriculture Organization (FAO)
- World Health Organization (WHO)
- Organization for Economic Cooperation and Development (OECD)
- International Life Sciences Institute. (ILSI)

Concerns about genetically modified plants / crops

- Societal / social concerns- is it required, will it help
- Environmental Concerns- immediate environment, Biodiversity
- Human and Animal Biosafety concerns- Toxicity, Allergy, unintended effects

Basic principle for safety assessment

- The comparison of a GM crop to its most closely related conventional counterpart

Is the product of an inserted gene/s harmful when consumed by humans or animals

- Nature of the new gene / new protein- Toxin/ enzyme/nutrient/ suppressor / gene silencing etc etc

- Understand its basic biology or method of action eg Bt protein / Cry proteins

Test for Toxicity/ Allergenicity
The Acute Tox study

- Pure protein gavage feeding to Rats
- Limit dose of 2000 mg / Kg B wt
- 14 days observation for mortality and morbidity
- Protein produced using bacterial expression system and confirmed for functional homology with native protein

Evaluation of potential allergenicity of novel proteins in transgenic food crops

The Protocols

- The IFBC-ILSI and FAO/WHO guidelines

Tests for Allergenicity- 1

- Amino Acid sequence homology with any allergen from database- 8 amino acid segments
- AA1 to AA8
- AA2 to AA9
- AA3 to AA10
- AA4 to AA11 and so on…….
- < 35% homology over a 80 AA stretch – low probability of allergen

Tests for Allergenicity- 2

- Protein digestibility -
- Resistant to digestion ? Allergen

- Thermal stability
- Resistant to heat - ? Allergen

- If any of the above 3 tests are positive – unlikely that the transgene would be approved
- Additional serological tests can be done

Other studies

- Ig E binding to GM protein- ELISA using hyperimmune human serum- if Allergen match is >35% and source of gene has H/o being allergenic
- Double Blind Placebo Controlled Food Challenge studies in hospital settings

Status

- To date there is no documented proof that any approved, commercially grown GM crop has caused allergic reactions owing to a transgenically introduced allergenic protein, or that generation of a GM crop has caused a biologically significant increase in endogenous allergenicity of a crop (Goodman 2008)

Substantial Equivalence

(DBT guidelines; ILSI- IFBiC- Argentina 2013)

Unintended effects

- By the insertion of defined DNA sequences(intended), additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects).
- **It is an inherent and general phenomenon and not specific to GMOs**
- Can occur more so in conventional breeding or mutation breeding.

COMPOSITIONAL ANALYSES OF KEY COMPONENTS

- Key nutrients or key anti-nutrients of those components in a particular food that may have a substantial impact in the overall diet.

- Major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as anti-nutrients)

- Minor compounds (minerals, vitamins).

Normal Variation In Composition

- Wide variation in the composition of any crop (conventional or otherwise) is normal, unavoidable and NOT a safety concern in a balanced diet
- Variation is driven by: climate & microclimate, variation from location to location and year to Year
- Environmental variation – soil, pest pressure, sunlight, location within a field
- seed genetic variation
- The composition of 2 plants taken from the same field will vary compositionally due to variations in microclimate and microenvironment

Safety Assessment Approaches
Andrew Bartholomaeus
School of Pharmacy, University of Canberra-presentation from ILSi IFBiC, Argentina 2013

Purpose of Compositional analysis

- Assess how similar is the GM crop to what is considered to be the conventional “safe” crop

–Deviations outside natural variability may indicate need for further evaluation

Information required

- Literature from a range of standard cultivars that are in commercial production for the same purposes and grown in the same geographical areas as those typically found in the Indian market may also be provided for assessing the nutritional relevance of any unintended effect.

Trial sites

- The location of trial sites needs to be representative of the range of environmental conditions under which the plant varieties would be expected to be grown.
- The number of trial sites need to be sufficient to allow accurate assessment of compositional characteristics over this range.
- Trials have to be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature

Trial sites

- Each trial site is required to be replicated to minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety,

- Sampling of adequate number of plants and the methods of analysis need to be sufficiently sensitive and specific to detect variations in key components.

Test Material ?

- Appropriate analyses must be performed on all the parts of the plant that may be used as food in India.

eg: If the intended uses of a transgenic corn event include the oil and the meal, samples of both corn oil and corn meal should be analyzed for the appropriate nutrients

If there is a difference

- The statistical significance of any observed differences will be assessed in the context of the range of natural variations for that parameter to determine its biological significance.

- If the composition of the GM food is judged not to be nutritionally equivalent to that of its parent and commercial varieties, i.e., significant differences (statistical and biological) exist in the nutrient data, **additional nutritional data may be required on a case-by-case basis**.

Nutritional Modified Plants

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include:

- 1. Nutrient intake recommendations;

- 2. The role of the food in the diet of the population;

- 3. The role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

Nutritional Change- intended or unintended

- The first phase of nutritional evaluation will be based on the nutrient composition data.
- If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis and assessment.
- **The safety of a major increase in the level of a nutrient** or other bioactive component would need to be assessed in a similar way to the safety assessment of an intended nutritional change

INTENDED NUTRITIONAL MODIFICATIONS

- Estimate the **likely intake of the food** derived from the GE plant.
- The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption.
- Basing **the estimate on the highest likely consumption** provides assurance that the potential for any undesirable nutritional effects will be detected.

INTENDED NUTRITIONAL MODIFICATIONS

- Impact of consumption **on specific population groups -** infants, children, pregnant and lactating women, the elderly and those with chronic diseases or compromised immune systems.

- Ascertain to what extent the modified nutrient is **bioavailable and remains stable with time, processing and storage.**

INTENDED NUTRITIONAL MODIFICATIONS

- The intended modification in plant constituents **could change the overall nutrient profile of the plant** product and this change could affect the nutritional status of individuals consuming the food.
- Unexpected alterations in nutrients could have the same effect.
- Although the GE plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile needs to be determined.

Feeding Studies

- **SUBCHRONIC FEEDING STUDY IN RODENTS**

- **Livestock feeding Studies**

SUBCHRONIC FEEDING STUDY IN RODENTS

- When compositional equivalence cannot be established
- There is uncertainty over the nutritional and/or health impacts of the difference;
- If the genetic modification affects multiple metabolic pathways and the potential impact on nutrition is not readily predictable;

SUBCHRONIC FEEDING STUDY IN RODENTS

If the genetic modification results in changes in levels of non-protein metabolites, or the synthesis of new ones;

or if other data are insufficient for a complete safety assessment.

90-day feeding study in rodents

- If animal feeding studies are warranted, it is recommended that a **90-day feeding study in rodents** be performed as the minimum to demonstrate safety

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- In order to avoid introducing adverse effects not directly related to the material itself, **the nutritional value and balance of the diets** used must be considered.

90-day feeding study in rodents

- **Chosen dose level should be one that does not cause nutritional imbalance**
- Should be **comparable to anticipated human intake**.
- **Not intended to assess the potential toxicity of the protein expression** product(s) of the inserted gene(s) as this is accomplished via the 14-day acute oral toxicity study in rodents.

Parameters

- Clinical
- Biochemical
- Hematological
- Gross Pathology
- Histopathology

Discussion and Interpretation of Results

- The significance and likely impacts of any abnormal findings should be discussed.
- **Where there are statistically significant differences in parameters between test and control groups, these should be discussed in terms of their biological significance and impact on safety.**
- The need, or not, of any additional or follow up studies should be discussed.

LIVESTOCK FEEDING STUDY

(to evaluate the nutritional parameters (*e.g.*, wholesomeness and nutrient bioavailability)

- Cattle, Goats, Poultry, Fish etc
- If there are significant and unequivocal compositional differences
- If the component of the GE plant given to the animals are a part of their natural feed
- Nutritionally modified plants

Feeding studies are not Tox studies

- Livestock feeding trials are **not designed, nor are they sufficiently sensitive, to evaluate the potential toxicity** of individual proteins or the potential toxicity associated with the whole food.

Choice of animal species

- Can be used in relatively large numbers to increase the statistical power of the study
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- Can be obtained as a nearly genetically uniform population in order to negate any effects of genetic background

- Is sensitive to the effect of small changes in nutritional quality on its growth and performance. **(eg Broiler Chicken)**

STUDIES TO BE COMPLETED BEFORE INITIATING BRL-1 TRIALS

STUDIES TO BE COMPLETED	Food & Feed Safety Assessment			Environmental Risk Assessment		
	Before first field trial	Field studies	Non-field studies*	Before first field trial	Field studies	Non-field studies*
Description of the genetically engineered plant						
Biology of the non-transgenic host plant						
Donor organism information						
Bioinformatic analysis: potential toxicity and allergenicity						

*run concurrently with field trials

Contd/-

Recommendations for staged completion of specific information and data requirements for the safety assessment of GE plants

STUDIES TO BE COMPLETED	Food & Feed Safety Assessment		Environmental Risk Assessment	
	Field studies	Non-field studies*	Field studies	Non-field studies*
Acute oral safety limit study				
Pepsin digestibility assay				
Protein thermal stability				
Subchronic feeding study in rodents (if required)				
Livestock feeding study (if required)				
Molecular characterization				
Inheritance of introduced trait				
Stability of introduced trait				
Expression of introduced protein(s)				
Compositional analysis				
Reproductive and survival biology				
Impact on non-target organisms: Tier I testing				
Impact on non-target organisms: Tier 2 testing				

*run concurrently with field trials

Biosafety research level I trials (BRL I)

- Biosafety Research Level I trials are limited in size to no more than 1 acre (0.4 ha) per trial site location and a cumulative total of 20 acres (8.1 ha) for all locations for each plant species/construct combination (e.g., one or more events originating from transformation of a plant species with the same genetic construct), per Applicant,per crop season.

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Biosafety Research Level II trials

- Biosafety Research Level II trials are limited in size to no more than 2.5 acres (1 ha) per trial site location and to no more than eight (8) locations within India for each plant species/construct combination (e.g., one or more events originating from transformation of a plant species with the same genetic construct), per Applicant, per crop season

Present status of pre approval testing in India

- The tests undertaken in private testing labs, contract research organisations and national institutions are accepted by regulatory agencies

- The private testing labs and CROs are either accredited by National Accreditation Board for Laboratories or GLP Compliance Committee under DST

Thank You