Preclinical Toxicology of GM Crops

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Status of genetic modification of crops

Existing: Simple modifications		Future: Complex traits	
Traits	**Crops**	**Traits**	**Crops**
Insect resistance	Maize, soybean, cotton	Nutritional modification	Rice, soybean, maize, canola
Herbicide tolerance	Maize, soybean, cotton, canola	Reduction in allergens/ anti-nutrients	Rice, maize, peanut
Virus resistance	Papaya, squash	Enhanced tolerance to biotic and abiotic stresses	Rice, cotton, maize
Insect resistance & herbicide tolerance	Maize, cotton, soybean	Enhanced shelf life/ processing properties	Potato, tomato

GM crops research in India: 22 Public & 10 Private sector Institutes

Type of food crops (17)	Rice*, maize, wheat, legumes, mustard*, groundnut, cotton, brinjal*, tomato, cauliflower, cabbage, potato*, musk melon
Traits targeted (6)	Pest & Disease resistance, herbicide tolerance, abiotic stress tolerance, nutritional enhancement, delayed ripening
Genes inserted (25)	Bt Cry genes, viral coat proteins, bar, chitinase, OXDC, Annexin, CodA, Ama1, Ssu-maize psy ans ssu-tp ctrl, etc.

*Awaiting field trials/ clearance/ approval

Biosafety issues

Environmental issues:

- Effects on non-target organisms
- Transgene escape and ecological consequences
- Development of pest resistance

Food safety and human health issues:

- Inherent toxicity of novel genes/gene products
- Potential to express novel antigenic proteins/ allergenicity
- Potential for nutritional changes
- Potential for unintentional effects

Aim of biosafety: To evaluate harm and /or exposure to harm

What is risk? = Risk= Harm/Hazard x Exposure

Risk	**Probability of occurrence of adverse effect from a hazard.**
Hazard	**i) Intrinsic property of a substance or object (transgenic plant/transgene product** **ii) with potential adverse or harmful effects.**
Exposure	**i) Quantitative measure** **ii) of the extent to which given hazard is present in a particular dimension** **iii) e.g. human food chain, environment.**

Step by step biosafety assessment based on level of use

Level of use	Level of environ. contact	Type of Control
Contained	No contact	Prevent potential exposure hazard poorly understood
Confined environ Release	Transitory, No durable presence	Prevent exposure
Unconfined Environ. release	Potentially durable presence	Hazards well understood Strict compliance with biosafety rules to prevent exposure

International frameworks and methodologies for assessing risks:

Cartagena Protocol on Biosafety

International plant protection Convention

OECD

UNEP

Codex

Setting up the framework for risk assessment of GM crops/foods

Define problem

Screen and prioritize risks

Consider management options

Testing for acute toxicity- Principle form of toxicity assessment of rDNA derived foods:

Basic concepts:

Provides information on possible health hazards from dietary exposure to novel proteins.

Carried out when there is no history of (safe) consumption of the new protein in human diet.

Principle focus on protein expression product of inserted gene.

Based on the concept that proteins exhibit toxicity via acute mechanisms in short time frame.

Testing for acute toxicity- Basic protocol

Follow OECD guidelines for testing of single chemicals.

Test substance: Bacteria/yeast/plant-derived recombinant protein expressed by the inserted transgene.

Test species: Standard laboratory mammalian test species (rat, mouse,).

Dose: High dose of several orders of magnitude above expected human exposure level.

No-observed adverse effect level (NOAEL): Levels at which adverse effects are not present.

Identify any potential adverse effects of importance to humans.

Establish safe upper limits for humans - Application of safety factors.

Sub-chronic toxicity testing: 90-day toxicity with Whole GM crop/ food:

Carried out under conditions when:

No substantial equivalence established between GM and non-GM food (molecular, compositional, phenotypic, agronomic,).

Alterations in metabolic pathways due to GM.

Potential impacts on nutritional status.

Alterations in level of non-protein metabolites/ synthesis of new compounds.

Information obtained:

Minimum test to assess safety of long-term consumption of GM foods.

Effects of repeated exposure over prolonged period of time from post weaning maturation and growth to adulthood.

Information on immunological, reproductive and neurological effects and unintentional effects.

Aids in defining further toxicity tests if potential for reproductive, developmental or chronic toxicity indicated.

Basic protocol:

Test duration of 90 days.

Test species: rats/ livestock (chicken, cattle, goats)

Test material: Edible part of the plant, whole GM plant/food.

Level of feeding: At levels without causing nutritional imbalances.

List of tests for evaluating toxicity and allergenicity potential of GM foods:

Toxicity:

Chemical Analysis for toxicants inherent to plant: Observe for alterations in levels.

Bioinformatic analysis for assessing potential homology of the expressed substances to known toxicants.

In vivo toxicity tests of expressed substances in defined/standard laboratory animal species:

Acute/ subchronic/ long term feeding studies.

Specific case studies: Reproductive, developmental, teratogenic and carcinogenic studies

Allergenicity:

Primary consideration:

Prevention of unexpected and unavoidable exposure of sensitized individuals to food allergens.

Assessment :

Potential for foods containing novel proteins to cross react to known food allergens.

Potential to lead to *de novo* hypersensitivity.

Potential to increase endogenous allergens due to GM.

Existing international guidance:

- Weight of Evidence Approach
- No single test can reliably predict an allergenic response.
- Further Improvements in bioinformatics approaches and experimental animal models.

Assessment of allergenicity potential: Basic strategy

1. Source of protein (allergenic?): Databases
2. Amino acid sequence homology: Bioinformatics / computer algorithms
3. Physico-chemical properties: Thermal Resistance/ resistance to digestive enzymes.
4. Serum screening for IgE cross reactivity:

   -Targeted serum IgE testing: Screening of serum samples from population allergic to the food group.

   - Specific serum testing: IgE binding to test protein from sera of individuals with known allergies to the source of the novel protein.
5. Level of protein in the food/plant

THANK YOU