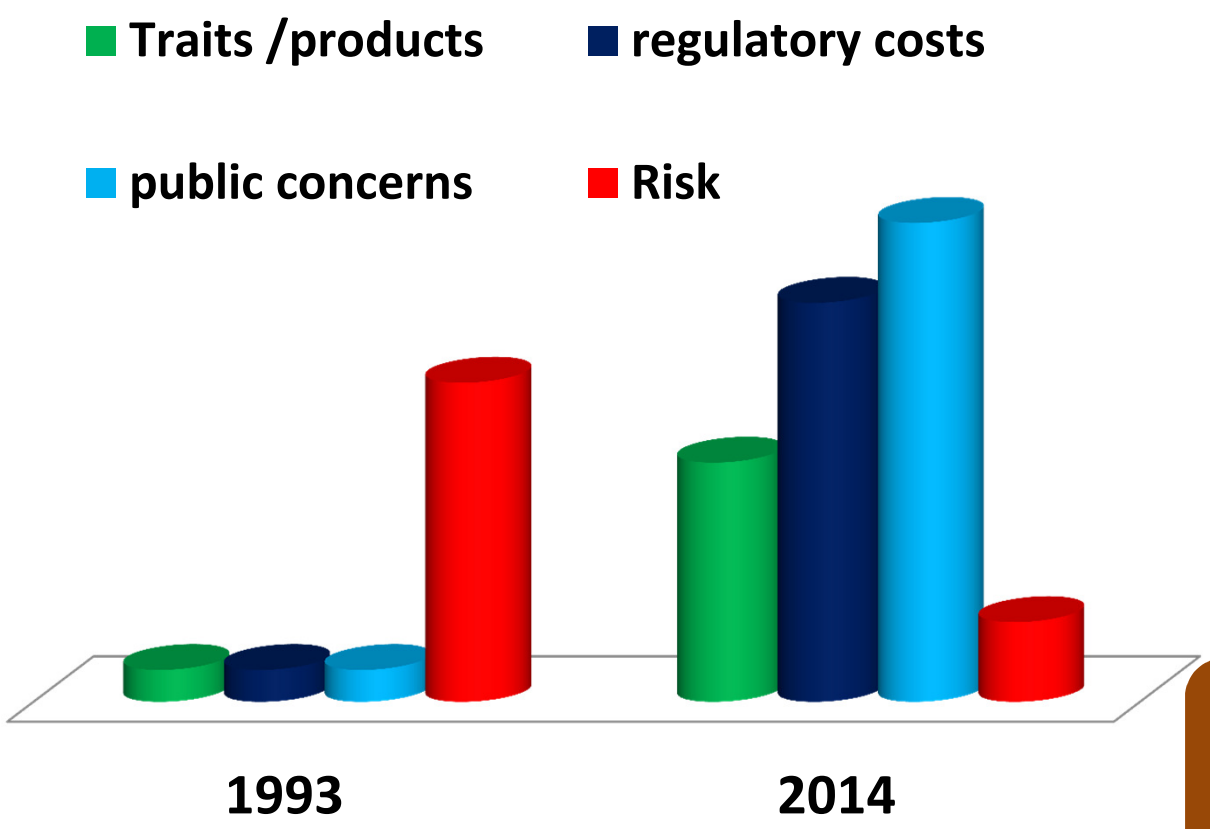
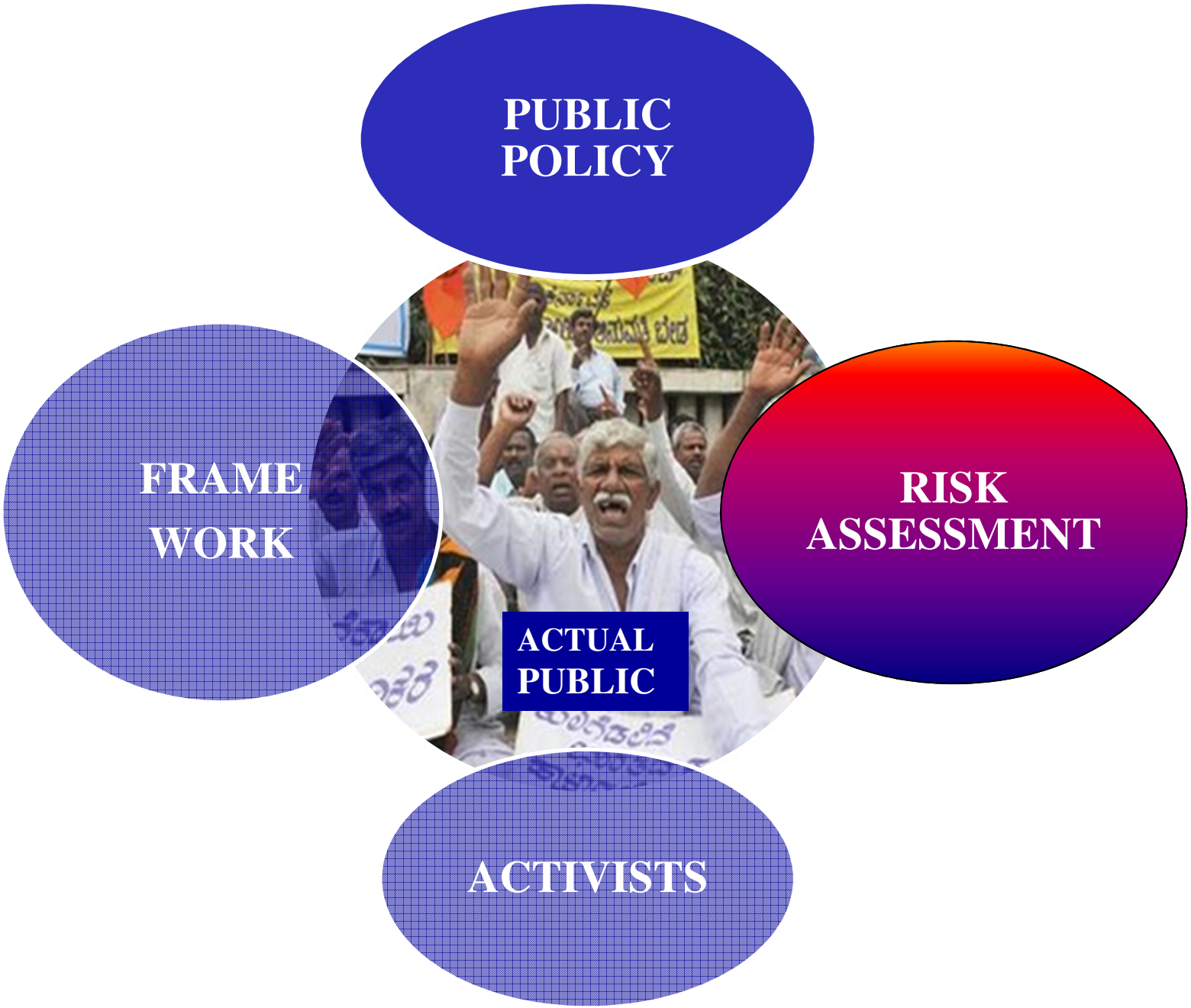
Regulatory Science in Agriculture Biotechnology – An Overview



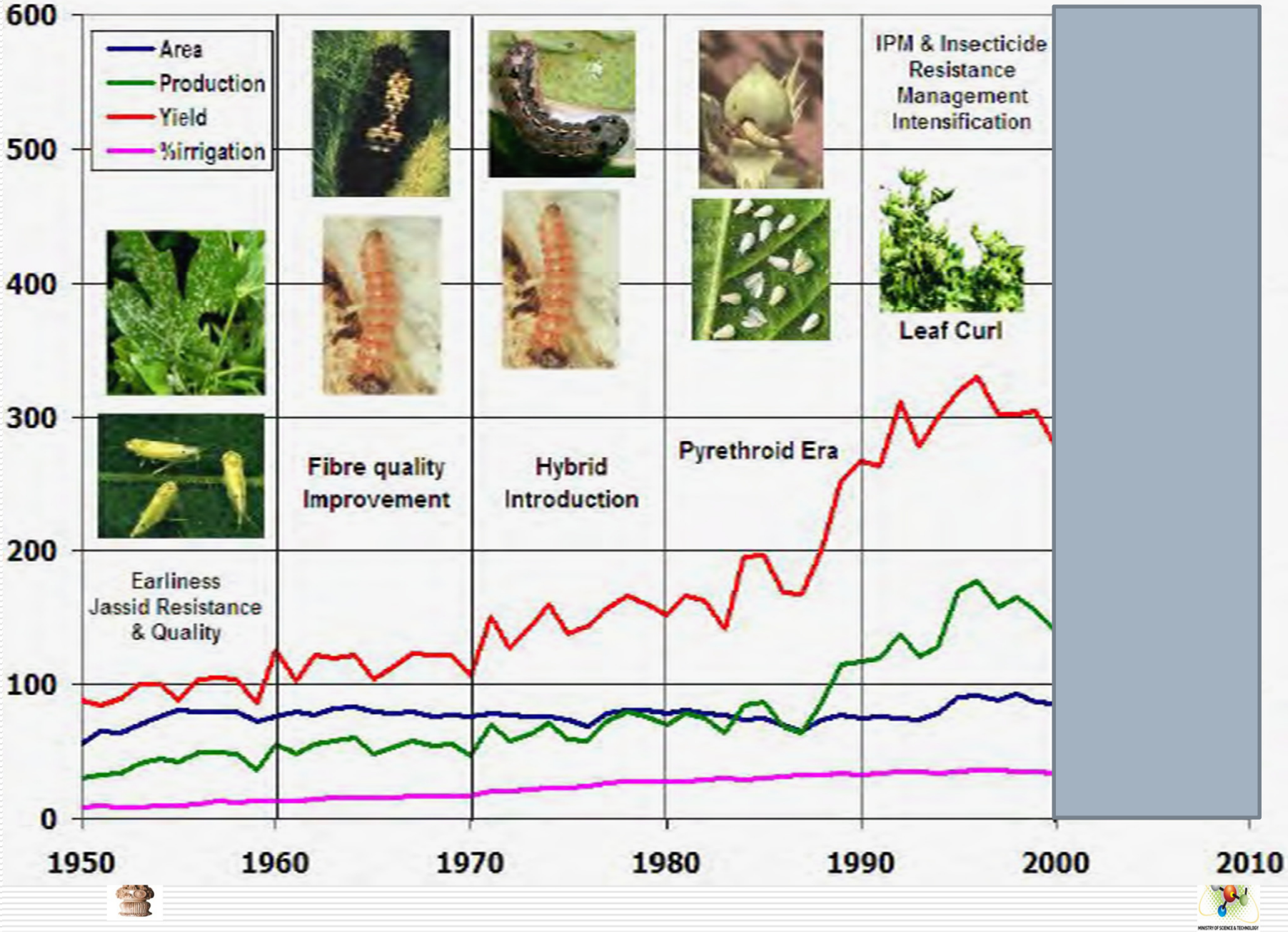
S.R.Rao

Dept. of Biotechnology
Government of India

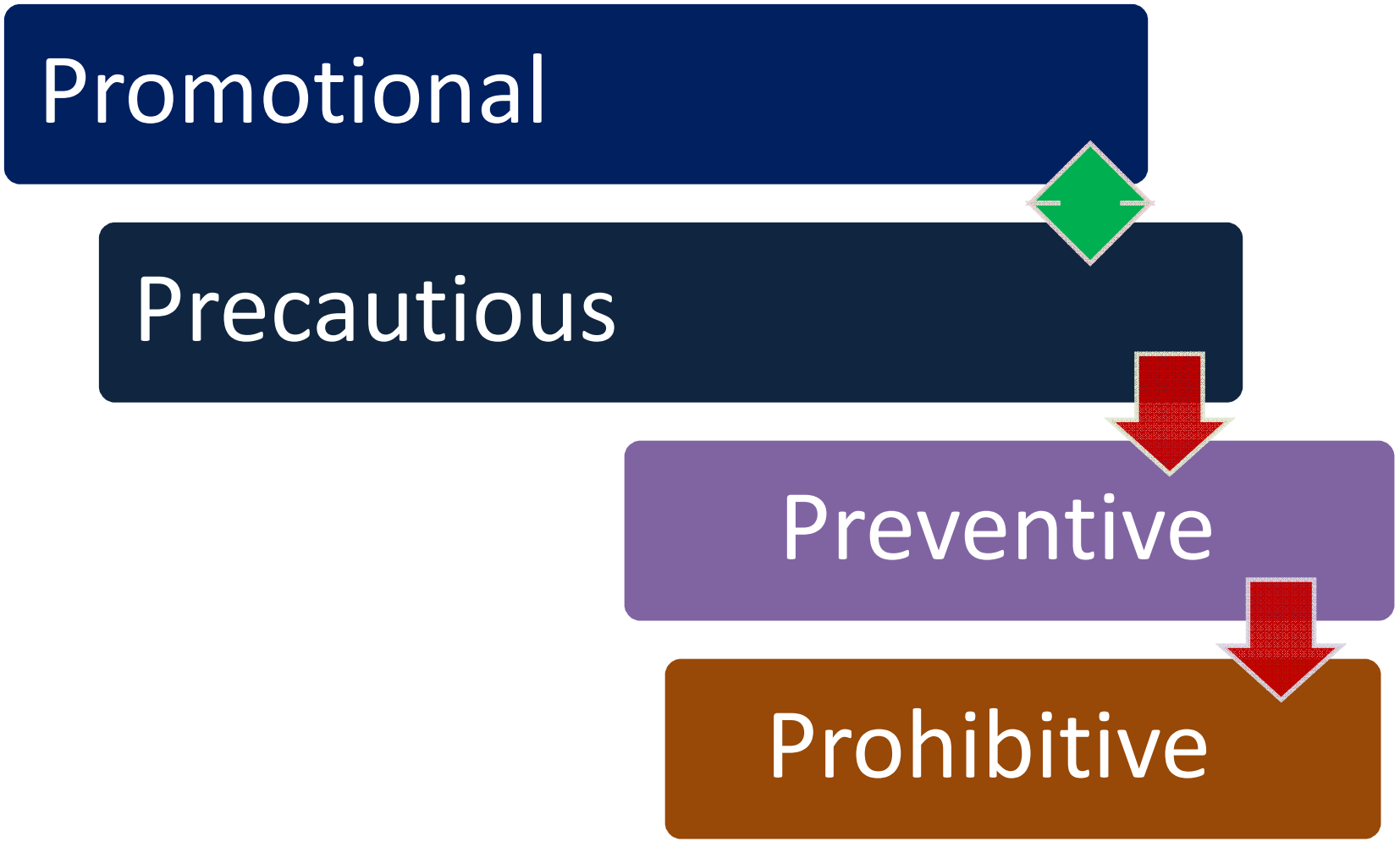
Expert Consultation On Regulatory Science For Risk Assessment In Agriculture Biotech , July 2o14, New Delhi


PUBLIC POLICY
FRAME WORK
RISK ASSESSMENT
ACTUAL PUBLIC
ACTIVISTS

Impact of Technology on Cotton in India



POLICY IN DIFFERENT COUNTRIES



Goals and Impacts for future technology

Technology helps address sustainability, food security & public

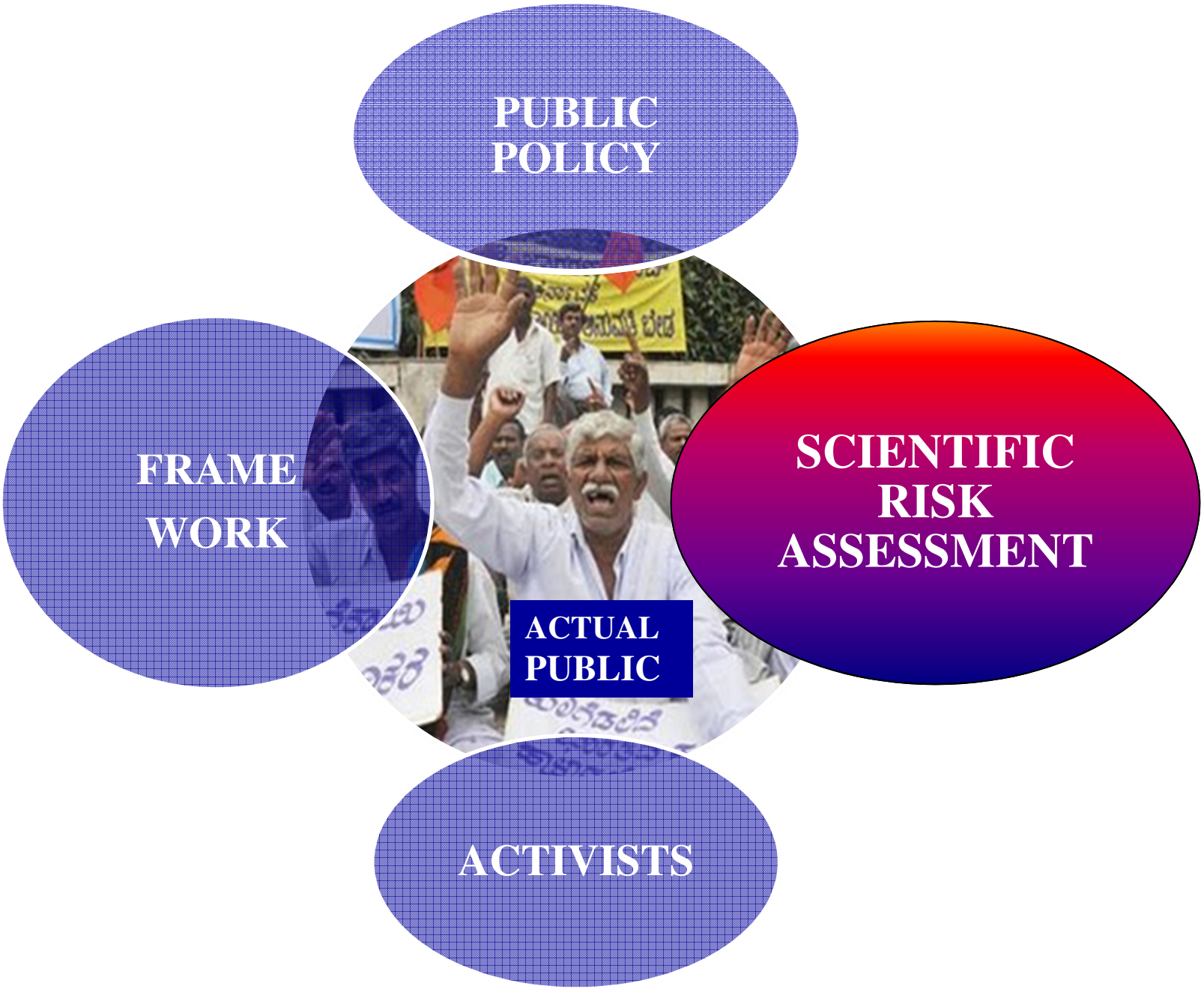
- Increased food production and improved nutrition
- Crops tolerant to biotic and abiotic stresses
- Reduced labor and costs

Technology helps contribute to environmental protection:

- Targeted use of crop protection products
- Reduced environmental footprint (land, water, nutrients, waste, etc…)
- Preserves and protects biodiversity

Ability to quickly respond to tomorrow's global challenges:

- Reduced product development time
- More targeted and precise breeding process


PUBLIC POLICY
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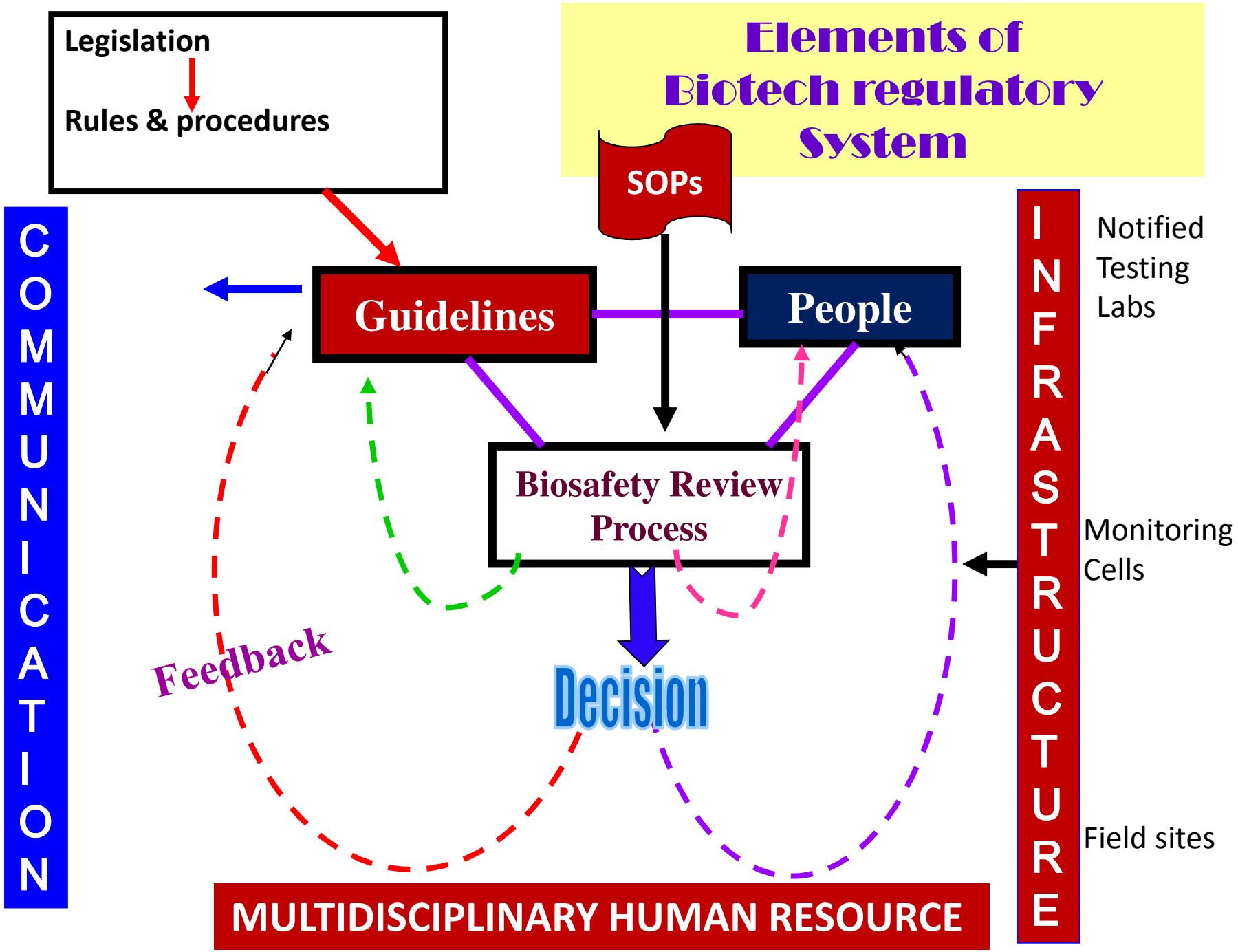

Elements of Biotech regulatory System
Legislation
Rules & procedures
SOPs
Guidelines
People
Biosafety Review Process
Decision
Feedback
COMMUNICATION
INFRASTRUCTURE
Notified Testing Labs
Monitoring Cells
Field sites
MULTIDISCIPLINARY HUMAN RESOURCE

The approach national level like international best practice followed for review or revision of protocols, guidelines of safety assessment of GM crops or any other global issues of that kind is to examine all the available peer reviewed research publications and documented experiences followed by wide ranging consultation at multiple level of stakeholders to arrive at consensus documents for wider adoption and harmonisation of practices at global level

Regulatory science, which is applied in nature, generates and makes use of evidence-based knowledge to facilitate decision-making about the safety, efficacy, quality and performance of products of modern biotechnology by developing and relying on new tools, methods, standards and approaches.

End points are clear . No However……

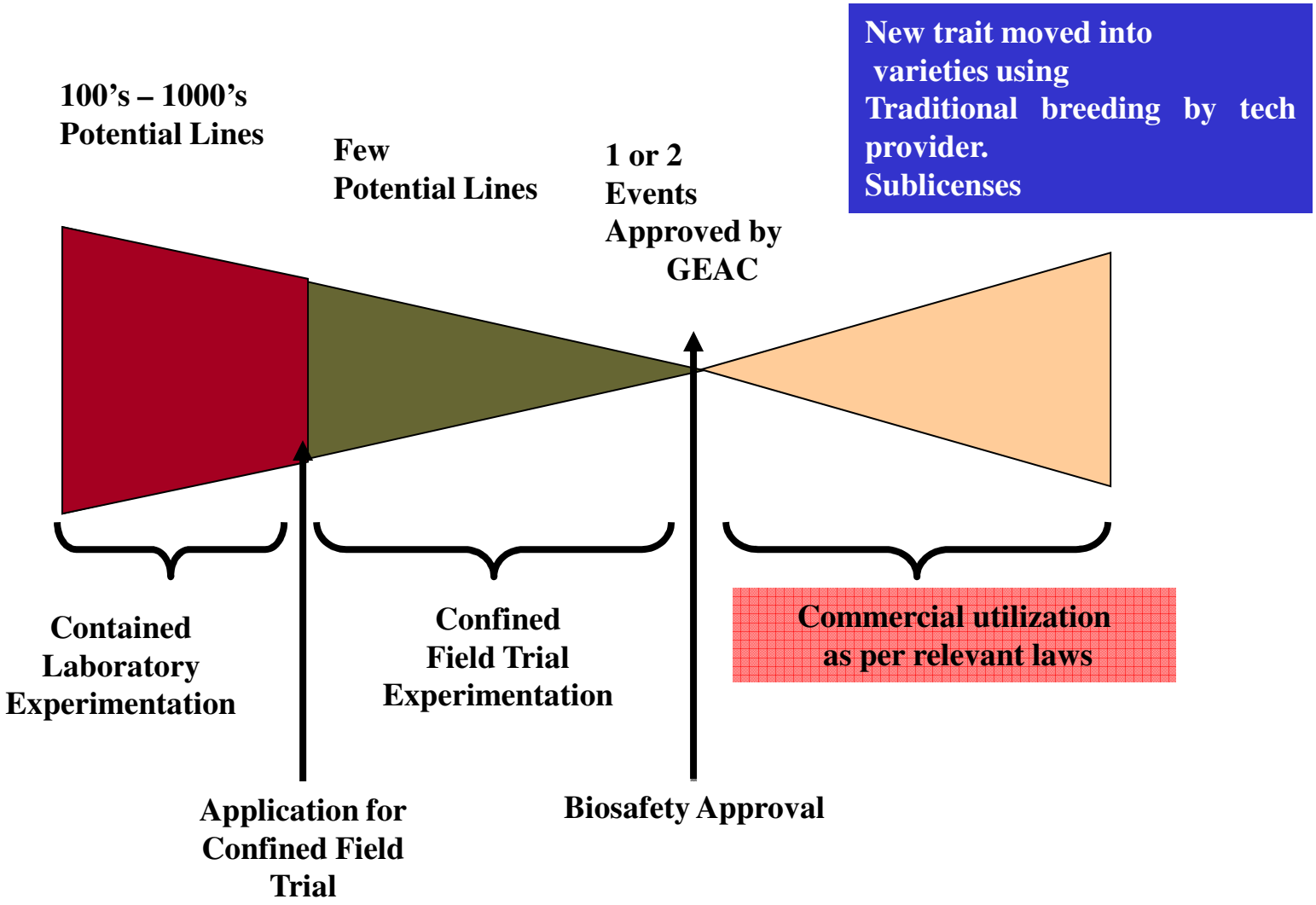
Regular science is gathering systematic knowledge of the physical or material or biological world gained through observation and experimentation. any of the branches of natural or physical science biological sciences etc. Around certain principles .

No end points . However……

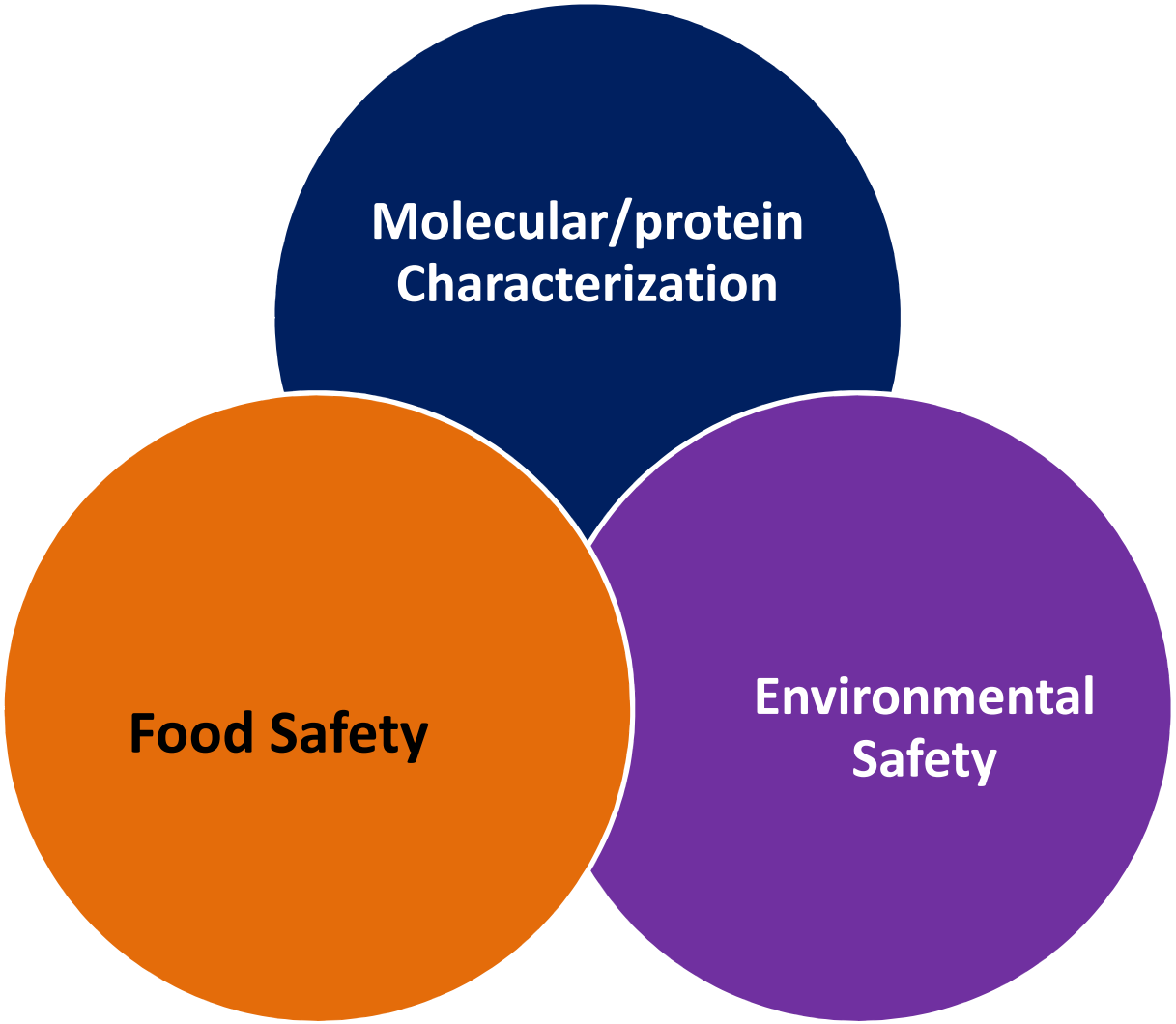
EVENT BASED APPROVAL SYSTEM OF A GE PLANT

Extensive safety assessment required;

only limited lines under confined field trials are finally approved



NATURE OF SAFETY ASSESSMENT REQUIREMENTS



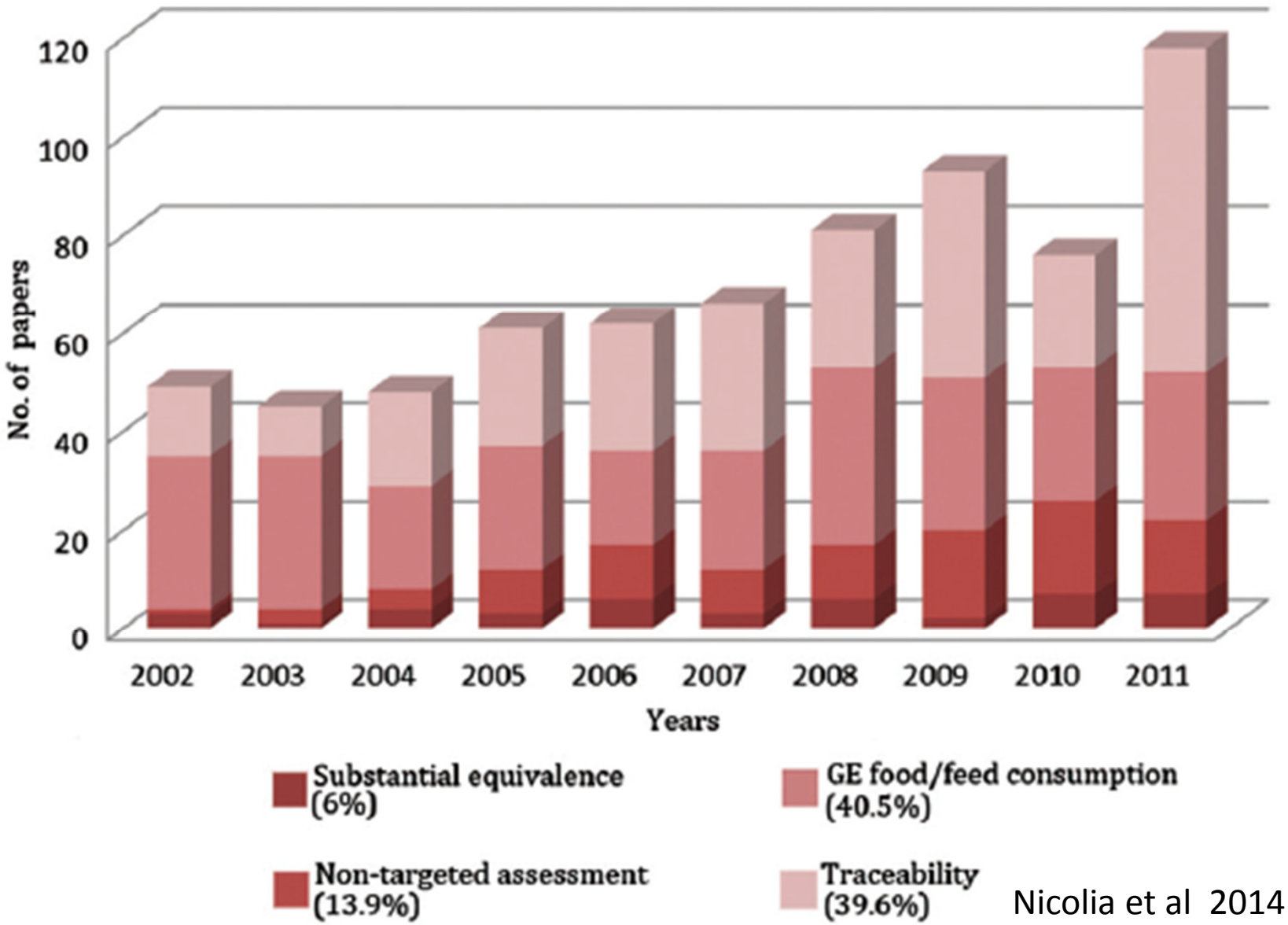
Food and feed safety assessment and the **environmental risk assessment** are **separate and distinct evaluations** and share some common elements of information through the **molecular characterization** of the GM organism and characterization of expressed, transgenic proteins

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

Publications out of 1783 total on GE food and Feed

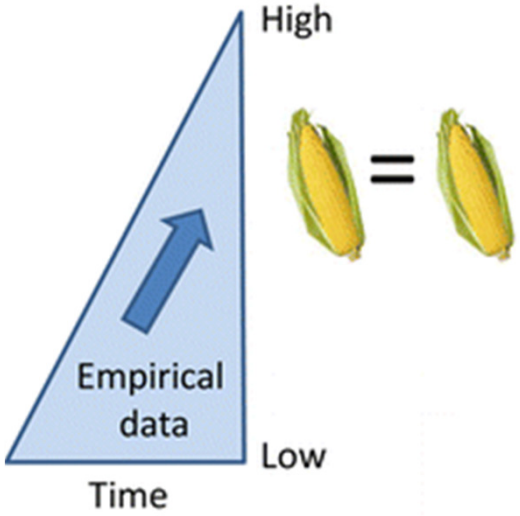


Nicolia et al 2014

Substantial Equivalence and food Safety

Key principle

A GM food will be considered to be *"substantially equivalent"* to the natural product if after a comparison of several different characteristics, no difference is shown



Criticism

- **Unexpected substances may appear in GM foods**
- ***GM foods approved on the basis of substantial equivalence are not safe because not tested rigorously enough***
- **Safety assessment based on "SE" not scientifically based**

GM Food Safety Evaluation

Category of Information/Data Requirement	India	Argentina	Australia	Canada	Philippines	Japan	S. Africa	EU	USA
Host information	X	X	X	X	X	X	X	X	X
Donor information	X	X	X	X	X	X	X	X	X
Molecular characterization	X	X	X	X	X	X	X	X	X
Characterization of expressed protein	X	X	X	X	X	X	X	X	X
Nutritional composition	X	X	X	X	X	X	X	X	X
Potential toxicity of novel protein(s)	X	X	X	X	X	X	X	X	X
Potential allergenicity of novel protein(s)	X	X	X	X	X	X	X	X	X

MOLECULAR CHARACTERIZATION

- **Well defined requirements**
- **Consensus documents on molecular characterization of plants derived from modern biotechnology published by OECD in 2010.**
- **Requirements by Indian regulations are in line with the above.**

Questions:

➢**Flanking sequences and safety**

➢identification of **open reading frames (ORFs)** within the inserted DNA or created by the insertions with contiguous genomic DNA including those that could result in fusion proteins

ORFs and Safety

Bioinformatic assessment, all naturally occurring stop-to-stop frames in the non-transgenic genomes of maize, rice, and soybean, as well as the human genome, were compared against the AllergenOnline (www.allergenonline.org) database using the Codex criteria.

It was discovered that thousands of frames that exceeded the Codex defined threshold for potential cross-reactivity suggesting that evaluating hypothetical ORFs (stop-to-stop frames) has questionable value for making decisions on the safety of GM crops.

Young et al 2012

Cross-reactivity is considered a possibility if more than 35% identity over a length of 80 or more amino acids (35%/ P80aa) is shared between the protein and the allergen
codex 2009

Protein Toxicity

TOXICOLOGICAL TESTING OF AN INTRODUCED PROTEIN WITHOUT A HOSU IS NOT NEEDED:

- **The introduced protein is structurally/functionally similar to a family of related proteins that have a HOSU in food, based on bioinformatics analysis and literature review.**
- **The biochemical function of the introduced protein has been adequately characterized.**
- **The introduced protein is readily digested when incubated *in vitro* with simulated digestive fluids.**
- **The introduced protein is susceptible to inactivation and/or denaturation during normal processing (e.g. cooking) of foods produced from that crop, based on either *in vitro* heat stability studies or food processing studies.**

Protein Toxicity…….

THE FOLLOWING CHARACTERISTICS WOULD INDICATE THAT AN INTRODUCED PROTEIN WITHOUT A HOSU WOULD REQUIRE TOXICOLOGY TESTING:

The introduced protein is shown by bioinformatics analysis to be structurally/functionally related to proteins that are known to be toxic to mammals.

The biochemical function of the introduced protein is not sufficiently characterized to predict risks for mammals.

There is potential dietary exposure to the functionally active protein because it is not degraded by digestive fluids when tested *in vitro*.

Impact of heating on functional activity of introduced proteins and food processing enzymes.*

	treatment		treatment	
CP4 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	65–75°C; 30 min	Enzyme*	None detectable	EFSA (2009d)
2mEPSPS	65°C; 30 min	Enzyme*	None detectable	EFSA (2007b)
Phosphinothricin-N-acetyl transferase (PAT)	55°C; 10 min	Enzyme†	None detectable	Hérouet et al. (2005)
Glyphosate acetyltransferase (GAT)	56°C; 15 min	Enzyme‡	None detectable	Delaney et al. (2008b)
Cry1Ab	80°C; 10 min	Insecticide¶	None detectable	de Luis et al. (2009)
Cry1F	75–90°C; 30 min	Insecticide	None detectable	EFSA (2005d)
Cry3A	95°C; 30 min	Insecticide	None detectable	US EPA (2010)
Cry9C	90°C; 10 min	Insecticide	No loss of activity	de Luis et al. (2009)
Cry34Ab1/Cry35Ab1	60–90°C; 30 min	Insecticide	None detectable	EFSA (2007a)
Acetolactate synthase	50°C; 15 min	Enzyme§	None detectable	Mathesius et al. (2009)
β-Glucuronidase	60°C; 15 min	Enzyme‖	50% loss of activity	Gilissen et al. (1998)

Dilemma of Composition

Two Decades of Research Confirms that Transgenesis Is Less Disruptive of Composition Compared with Traditional Breeding

The merits of continuing to generally require compositional analysis of GM crops to inform safety seems dubious given the results of 20 years of research, and if agreement can be reached that these studies are no longer warranted, use of this technology will become accessible to a wider array of scientists. *Herman 2013*

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same

These studies have spanned the crops of corn, soybean, cotton, canola, wheat, potato, alfalfa, rice, papaya, tomato, cabbage, pepper, raspberry, and a mushroom, and traits of herbicide tolerance, insect resistance, virus resistance, drought tolerance, cold tolerance, nutrient enhancement, and expression of protease inhibitors

Points to Ponder on compositional analysis

- The purpose of compositional analysis in the light of the state of the knowledge about natural variability, genome plasticity and the experience with GM technology: Is it to identify unintended effects? Ensure nutritional content? Monitor toxins?
- Revisiting the purpose of compositional analysis may provide scope to reframe compositional analysis to focus only on critical nutrients and antinutrients for some trait/crop combinations instead of a full compositional dataset.
- There is a need to extend the composition databases to include local data and information on GM crops, new crops, and data obtained by alternative analytical methods.
- It would be helpful if Best Practices documents were developed on Quality Guidelines for Regulatory Sciences (vs GLP) for composition and other studies. This would greatly help local and public sector developers.

Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review……

- 12 long-term studies (of more than 90 days, up to 2 years in duration) and 12 multigenerational studies (from 2 to 5 generations)
- 90-day studies on GM feed for which long-term or multigenerational study data w
- Man ological
- exami ic DNA.
- The s udy.
- Resu and, in
- genera ameters
- observ
- How thin the

normal variation range of the considered parameter and thus had no biological or toxicological significance.

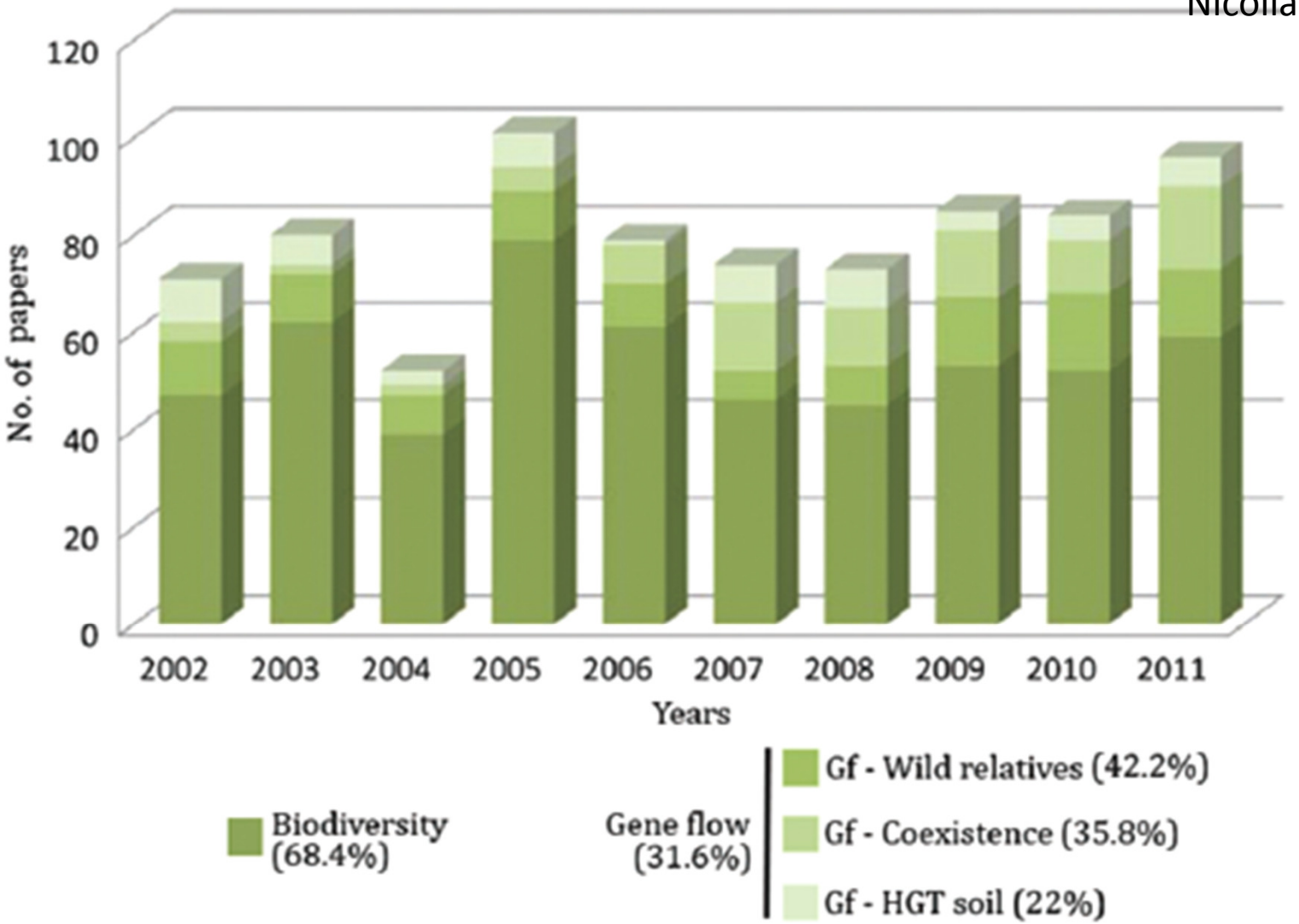
It was recognized that most studies are conducted more for the benefit of public perception than scientific benefit, but there are substantial ethical, reputational and risk communication issues involved with that approach. Better communication is needed to avoid studies conducted primarily for public perception.

- Seven with Bt Maize on chicken, cattle , goats sheep and one Rice on monkey

Chelsea Snell et. al (2012) Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. Food and Chemical Toxicology 50 (2012) 1134–1148

Main topics belonging to Environmental Risk Assessment

Nicolia et al 2014



Phenotype of the GE plant

Information/Data Requirement					
Growth habit	√	I	I	√	√
Life-span	√	I	I	√	√
Vegetative vigour	√	I	I	√	√
Ability to overwinter (or overseason)	√	I	I	√	√
Number of days to onset of flowering; number of days for flowering	√	I	I	√	√
Number of days until maturity	√	I	I	√	√
Seed parameters	√	√	I	√	√
Proportion surviving from seedling to reproduction	√	I	I	√	√
Outcrossing frequency (intra- and interspecific)	√	√	√	√	√
Impact on pollinator species	√	√	√	√	√
Pollen parameters		√	I	√	√
Fertility	√	I	I	√	√
Self-compatibility		I	I	√	√
Asexual reproduction	√	√	√	√	√
Seed dispersal factors	√	√	√	√	√
Symbionts		√	√	√	√
Stress adaptations	√	√	√	√	√
Add or subtracts substances to/from soil		√	√		

Marvin 2014

ENVIRONMENTEAL SAFETY ASSESSMENT

- Inter-country comparison of information requirements clearly indicates that there is a high degree of harmonization across countries in terms of what information and data should be considered in the context of environmental safety assessment.

- A working Group on Harmonization of Regulatory Oversight in Biotechnology working since 1995 on the information used in ERA and methods of analysis.

- 43 consensus documents produced so far on a range of issues.

INFORMATION REQUIREMENTS BEFORE PERMITTING CONFINED FIELD TRIALS

- **Prior to the conduct of field trials, the information to be provided by the applicant relevant to risk assessment includes:**
  - **Description of the GM plant**
  - **Description of the biology of the non-modified host plant**
  - **Description of the genetic modification(s)**
  - **Confirmation of inheritance of the new trait(s) over multiple generations**
  - **Assessment of possible toxicity and allergenicity by comparing amino acid sequence similarity of the newly expressed proteins with known protein toxins and allergens.**

- **This is consistent with guidance/requirements in various countries with active regulatory system viz. Australia, Brazil, USA, Canada etc.**

- **There is no pre-set requirements for safety studies (as indicated in their regulatory documents) and the focus is more on confined field trial management to restrict the spread of the regulated material.**

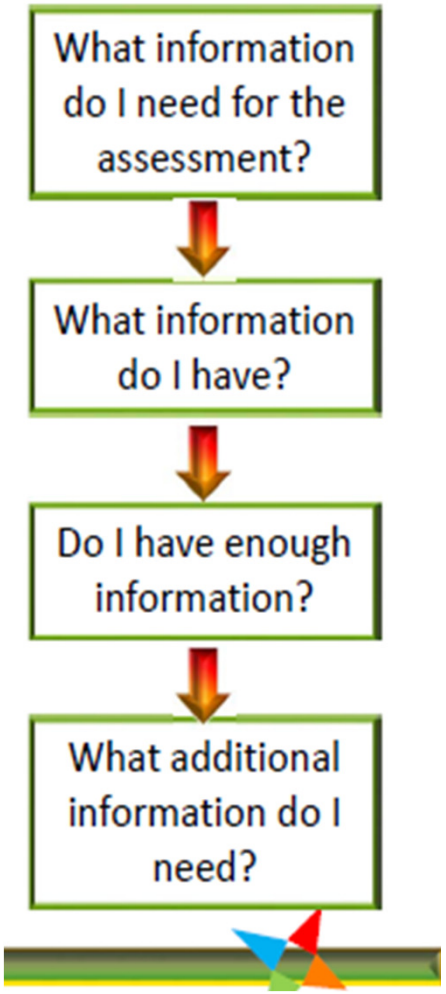
Table 2: Reported cross-pollination in brinjal in India

% Cross pollination	Reference
0.00-48.00%	Agrawal, R.L. (1980)
6.00-20.00%	Choudhary, B. (1971)
6.70%	Sambandam, C.N. (1964)
30.00-40.00%	Daskalov *et. Al.* (1937)
0.14-1.99%	Choudhary *et. al.* (1970)

The extent of cross pollination has been reported as high as 48 percent and depends on pollinating insects

Problem formulation is methodology that allows the organization of the risk assessment in logic way

- In this step the risk assessment is where the protection goals are clearly outlined as well as assessment end points
- Relevant available information is compiled to address key questions
- Facilitates an initial risk characterization to establish :
  - If the risk characterization can be completed with available information
  - If more information is necessary
- If more information is necessary , the problem formulation allows
  - The development of clear analysis plan
  - The identification of information needed to facilitate decision making



MINISTRY OF ENVIRONMENT & FORESTS
NOTIFICATION
New Delhi, The 5th December, 1989
RULES FOR THE MANUFACTURE, USE/IMPORT/EXPORT AND STORAGE OF HAZARDOUS
MICRO ORGANISMS/ GENETICALLY ENGINEERED ORGANISMS OR CELLS
(To be notified under the EP Act, 1986)

2. APPLICATION

(1) These rules are applicable to the manufacture, import and storage of micro-organisms and Gene-Technological products.

(2) These rules shall apply to genetically engineered organisms/micro-organisms and cells and correspondingly to any substances and products and food stuffs, etc., of which such cells, organisms or tissues hereof form part.

(3) These rules shall also apply to new gene technologies apart from those referred to in clauses (ii)and (iv) of rule 3 and these rules shall apply to organisms /micro-organisms and cells generated by the utilisation of such technologies and to substances and products of which such organism and cells form part.

These rules shall be applicable in the following specific cases:

(a) sale, offers for sale, storage for the purpose of sale, offers and any kind of handling over with or without a consideration:

(b) exportation and importation of genetically engineered cells or organisms:

(c)production, manufacturing, processing, storage, import, drawing off, packaging and repackaging of the Genetically Engineered Products:

(d) production, manufacture etc. of drugs and pharmaceuticals and food stuffs distilleries and tanneries, etc. Which make use of micro-organisms/ genetically engineered microorganisms one way or the other.

DEFINITIONS

In these rules unless the context requires.

(i) "Biotechnology" means the application of scientific and engineering principles to the processing of materials by biological agents to produce goods and services;

(ii) "Cell hybridisation" means the formation of live cells with new combinations of genetic material through the fusion of two or more cells by means of methods which do not occur naturally;

(iii) "Gene Technology" means the application of the gene technique called genetic engineering, include selfcloning and deletion as well as cell hybridisation;

(iv) “Genetic engineering” means the technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material;

(v) “microorganisms” shall include all the bacteria, viruses, fungi, mycoplasma, cell lines, algae, protozoans and nematodes indicated in the schedule and those that have not been presently know to exist in the country or not have been discovered so far.

Future plans Indian Biotechnology Regulation

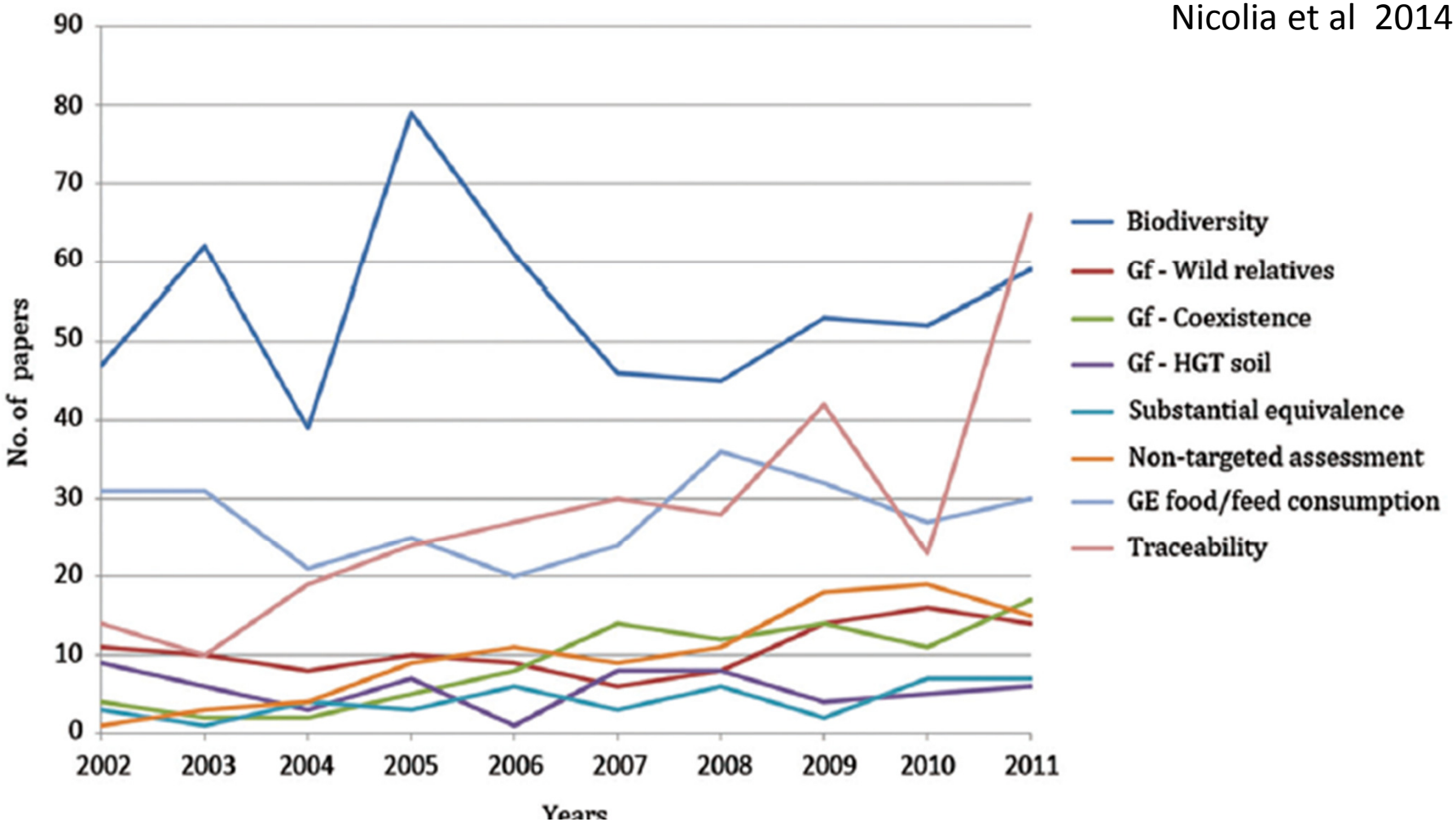
- **Establishment of central agency for regulatory testing and certification laboratories**
- **Promotion of regulatory science research units and HRD**
- **Centre for Biotechnology Communication**

Initiatives Proposed

1. **Establishment of a non-statutory unit to assist RCGM/GEAC**
2. **Creation of Regulatory testing facilities and up gradation of existing facilitates in public sector**
3. **Devising accreditation and notification systems for the laboratories engaged in Biotechnology Research & testing and notification of field trial sites**
4. **Funding Regulatory Science**

Growth of publications on various topics

Nicolia et al 2014



Drivers NPBT in Plant Breeding

Although NPBTs may be considered new:

- **NPBTs are innovative improvements and refinements of existing breeding methods**
- **Resulting products in many cases are indistinguishable/similar from existing products produced by traditional breeding techniques**
- **NPBTs enhance the efficiency and specificity of breeding, with more knowledge and understanding of the final product than ever before**
- **Adaptable to a variety of crops, including trees and vegetables, by researchers from all sectors (public and private, large and small)**

"NPBT"

Snap-shot of today's current, evolving breeding tools:

1. **ODM**
2. **SDN (-1,-2,-3)**
3. **Cisgenesis**
4. **Grafting**
5. **Agro-infiltration**
6. **RdDM**
7. **Reverse Breeding**

Example Cisgenics

Drivers:

- **Allows for rapid introduction of desirable traits between two breeding species (*sexually compatible*)**
- **Crop development can be reduced by decades**
- **Conventional breeding may result in the introduction of additional undesirable traits in final product *(linkage drag)***

Nature, 20 Aug 2013

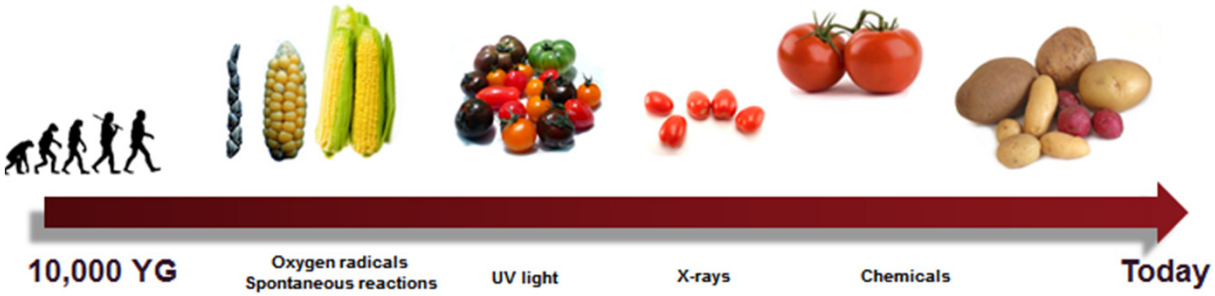
Example: Apple Scab

- **Took 85 years to conventionally breed scab resistant commercials apples**
- Fungus (*Venturia inaequalis*) has overcome resistance
- **Estimated with conventionally breeding it will take 40 years to breed in resistance**

- **Cisgenic traits can reduce the breeding process by 50% or more**
- Final product does not differ in any meaningful way from existing apple varities

Example: Mutations (ODM, SDN-1, SDN-2)

MUTAGENESIS IS FUNDAMENTAL TO EVOLUTION & CROP BREEDING-SELECTION

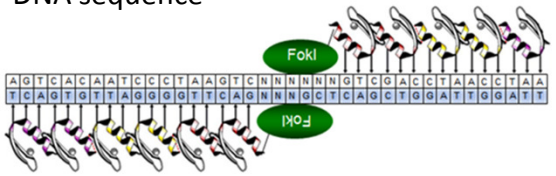


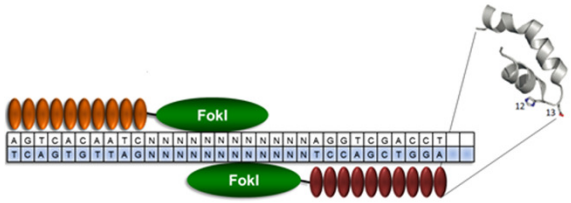
- Mutational products have a long history of safe use. Over 3,200 cultivars have been used commercially and are globally adopted
- SDNs continue the history of improving crop development through modern targeted mutational applications
- ODM/SDN-1/2 allow, for the first time, mutations to be targeted to a specific, desired location in the plant genome

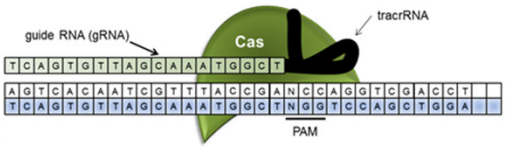
SITE DIRECTED NUCLEASES

1. **Zinc Finger Nucleases**
2. **Meganucleases**
3. **TALENs**
4. **CRISPRs**
5. ***ETC…***

DNA binding and restriction proteins which can be designed to recognize a specific DNA sequence







Products under Development

Cisgenics / Intragenics:

Apple scab resistance, potato late blight resistance, drought/cold tolerant maize, fungal resistant papaya, improved forage ryegrass, a variety of vegetable crops

Grafting:

Citrus trees with transgenic rootstock

SDN (-1/-2/-3):

Improved nutritional quality maize, higher yield tomatoes, disease resistant wheat, improved nutritional quality canola, nematode resistance

ODM:

Herbicide tolerant oilseed rape, herbicide tolerant flax

COUNTRIES

Argentina
Australia
Belgium
Canada
Ireland
Japan
Mexico
Switzerland
The Netherlands
United Kingdom
United States

CROPS

Apple
Canola
Cassava
Cereal Grains
Citrus
Flax
Maize
Papaya
Ryegrass
Tomato
Wheat

DEVELOPERS
USERS

SMEs
Academics
Industry
Non-for-profit

Perspective on ERA:

- **Techniques used to develop new plant varieties do not pose a specific safety hazard as witnessed by the long history of safe use of plant varieties produced through human domestication and breeding.**

- **It is the characteristics of the plant that determines its safety**

  The need to regulate/assess plants developed through NPBTs should be driven by the characteristics of the product rather than by the production method or process used to produce that product.

  For example:
  - *whether the product is materially different from existing products present in food, feed or the environment.*

- **Products developed through NPBTs are in many cases similar / indistinguishable to products developed through existing breeding methods**
  - *Products already have a long history of safe use*
  - *When applicable, products already have ERA in place*

- **As some NPBTs offer improved precision and enhanced understanding of the final products, NPBTs reduce questions around safety assessment, including the ERA of the final product**

Perspective on ERA:

Considerations for an ERA should be…

- **Driven by the characteristics of the product rather than by the production method or process used to produce that product.**

- **Based on the degree to which the product is creating new potential safety concerns**

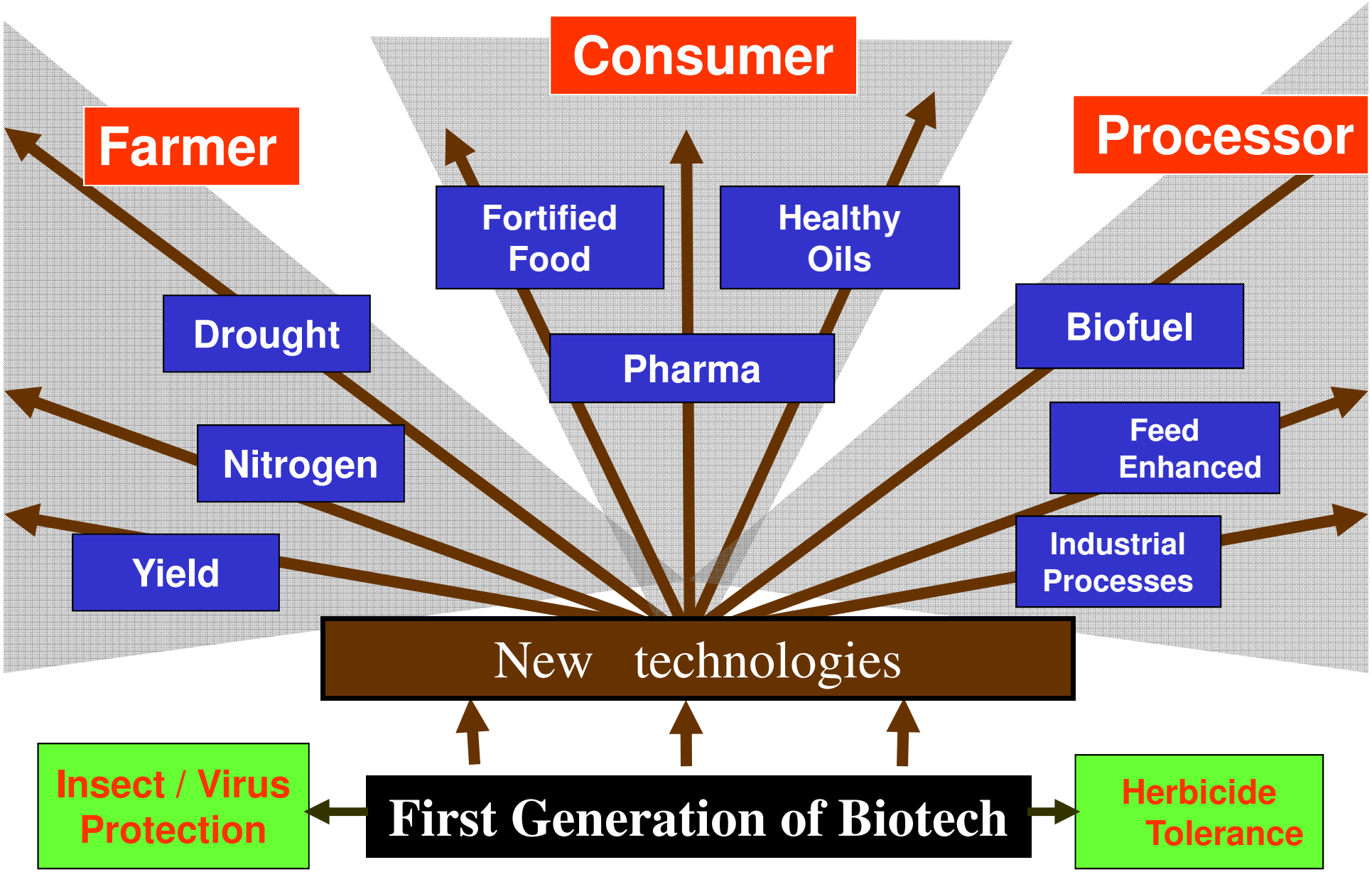
  Strong indicators for the absence of safety effects are…

  - ***The gene pool used in the process does not differ from that exploited in traditional breeding (sexual compatibility)***
  - ***The product / expressed trait has a history of familiarity***
  - ***Genome changes are so small that they are in the order of magnitude of what occurs naturally and in traditional breeding based on sexual compatibility (natural variability)***

- **Based on sound scientific principles** ***(need to know vs. nice to know)***

  e.g. Does the resulting product raise any additional concerns compared to products produced via conventional breeding for the environment or food/feed chain?

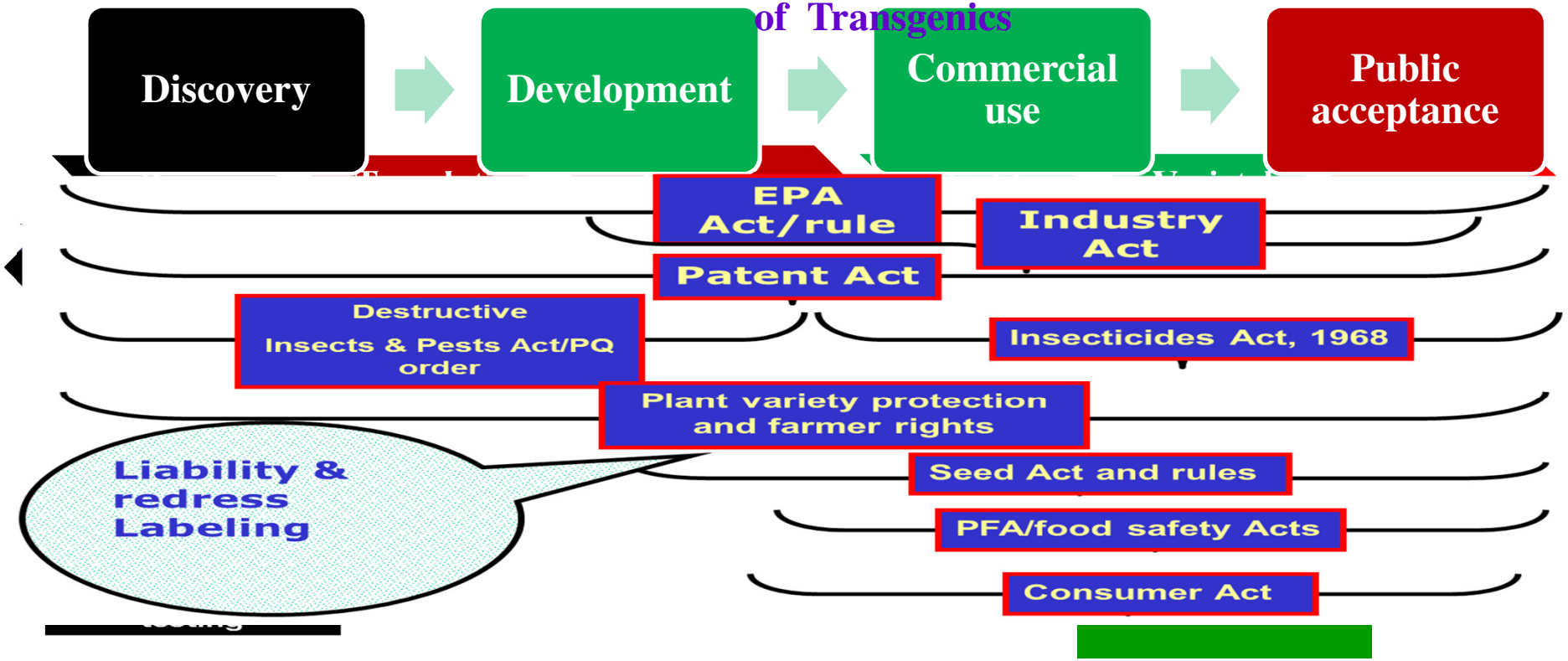
Future Generation of traits

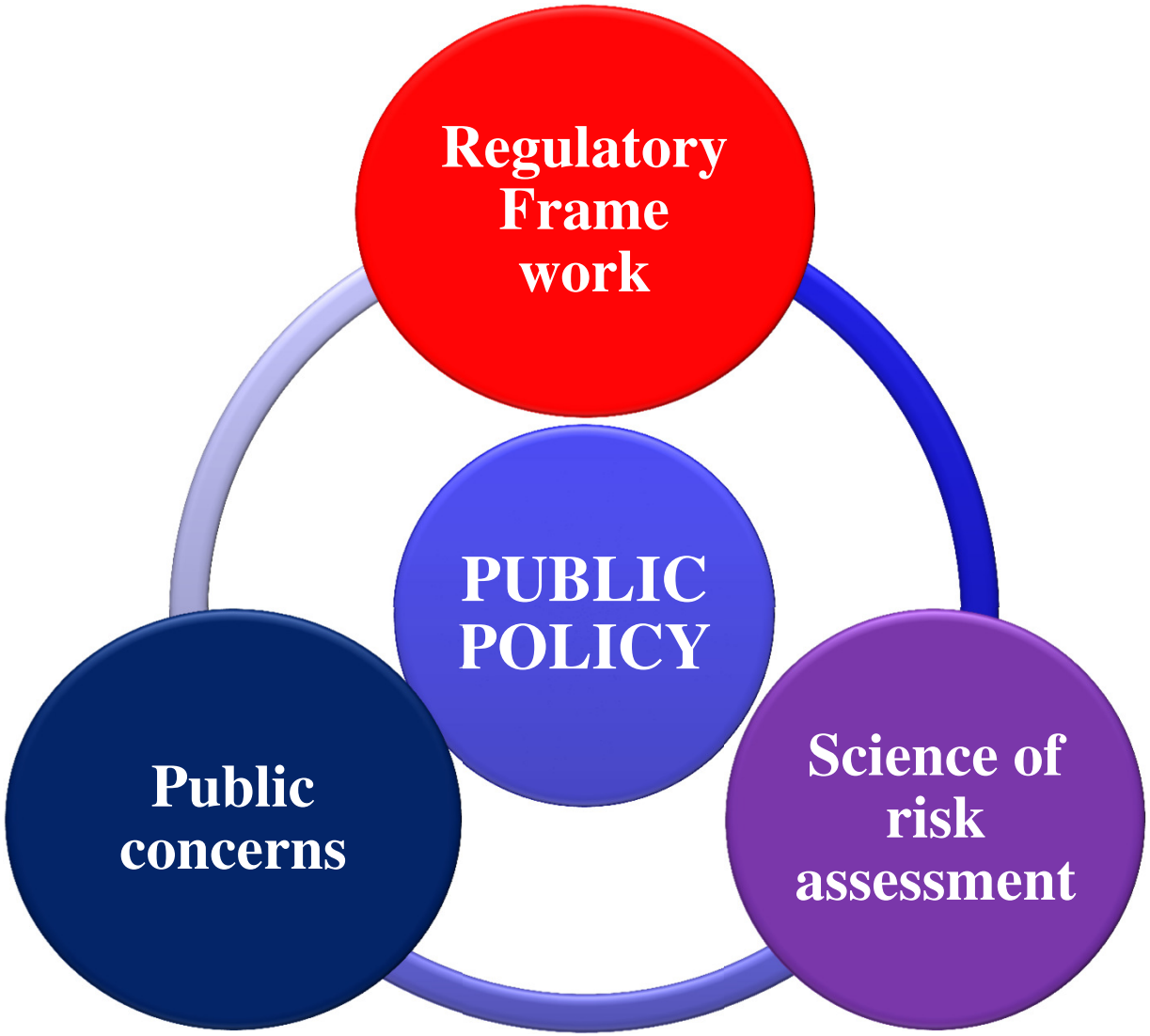


The Supreme Court of India, for the first time, on September 22, 2006, issued an interim verdict banning all field trials of genetically modified (GM) crops in the country and slammed its regulatory mechanism.

Supreme court constituted committee of five experts in Interim report recommended ban on field trials, heard and stayed for detailed report with induction of one more experts

Complexity with Research Development and Commercialization of Transgenics




Regulatory Frame work
PUBLIC POLICY
Public concerns
Science of risk assessment

Thanks