

# **Pre-Market safety of GM crops**

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## **Need for Safety assessment of GM plants**

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1. Novel or modified characteristics in GM plants
  2. Biosafety regulatory requirements
  3. International commitments

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## 1. Novel or modified characteristics in GE plants

A GE plant carries new gene(s)

New gene(s) can be introduced into a plant from any source - bacteria, virus, fungi, animals - thus overcoming taxonomic or reproductive barrier

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## 2. Biosafety regulatory requirements

- National requirements
  - Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells, 1989 (Rules, 1989) notified under the Environment (Protection) Act, 1986.
- International commitments
  - Cartagena Protocol on Biosafety

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### 3. Key International Forums for GMOs and biosafety discussions

Codex Alimentarius



Organization of Economic Cooperation and Development (OECD)



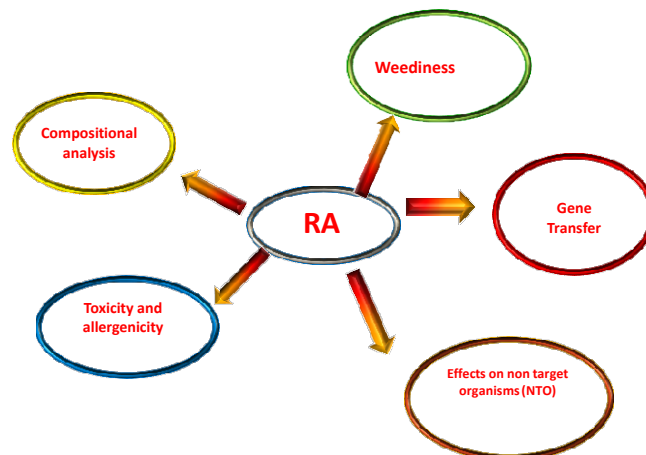
Cartagena Protocol on Biosafety to the Convention on Biological Diversity



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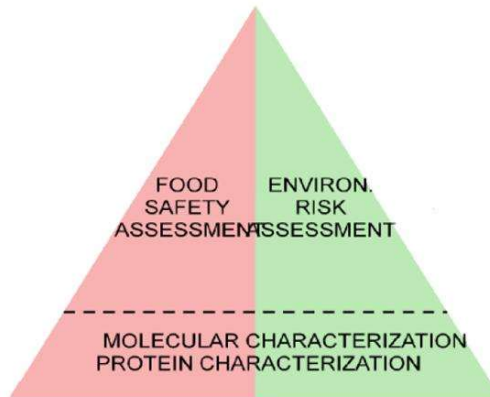
### Safety concerns/Areas of assessment



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## Components of safety assessment of GE plants



The food safety assessment and the environmental risk assessment of GM organisms are separate and distinct evaluations that share some common elements of information provided through the molecular characterization of the GM organism and characterization of the expressed, transgenic proteins.

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## Assessing food safety

- Foods are **complex mixtures** of macro- and micro-nutrients, fibres, anti-nutrients, natural toxins, and health-promoting substances
- Safety evaluation of whole foods as performed with single food additives is not possible – the concept of “**acceptable daily intake**” does not generally **apply** to food

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## A decade of consultation

- UN Food and Agricultural Organization (FAO)
  - 1991, 1996, 2000, 2001
- UN World Health Organization (WHO)
  - 1991, 1993, 1995
- International Life Science Institute (ILSI)
  - 1996, 1997
- Organization for Economic Cooperation and Development (OECD)
  - 1993, 1996, 1997, 2000
- International Food Biotechnology Council (IFBC)
  - 1990
- CODEX Alimentarius
  - 2003

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## Safety considerations

### ➤ Core Characterization

#### ➤ Gene(s)

- Source(s)
- Molecular characterization
- Insert / copy number / gene integrity

#### ➤ Protein(s)

- History of safe use and consumption
- Function / specificity / mode-of-action
- Levels
- Toxicology / allergenicity testing

### ➤ Food/Feed Composition

- Proximate analysis
- Key nutrients
- Key anti-nutrients
- Animal performance assessment

### ➤ Environmental

- Host organism
- Safety to non-target organisms
- Soil degradation, toxicity
- Outcrossing, weediness

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## Biosafety Guidelines for GE Crops

### Contained Use (DBT)

- Recombinant DNA Safety Guidelines, 1990 (Updated 2017)
- Revised Guidelines for Research in Transgenic Plants, 1998

### Confined Field Trials (MoEF&CC and DBT)

- Guidelines for Conduct of Confined Field Trials of Regulated GE Plants, 2008
- Standard Operating Procedures (SOPs) for CFTs of Regulated, GE Plants, 2008
- Guidelines for Monitoring of Confined Field Trials of Regulated GE Plants, 2008

### Food Safety Assessment (DBT and ICMR)

- Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, 2008 (Updated in 2012)
- Protocols for Food and Feed Safety Assessment of GE Crops, 2008

### Environmental Safety Assessment (MoEF&CC and DBT)

- Guidelines for Environmental Risk Assessment (ERA) of GE Plants, 2016
- Risk Analysis Framework, 2016
- ERA of GE Plants: A Guide for Stakeholders, 2016

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## GM crops approved/considered for environmental release in India

### *Approved GE plant in India (Bt cotton)*



*GE plants recommended for environmental release by GEAC in India (Bt brinjal and GE mustard); govt approval pending*



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# Preclinical Safety Assessment by ICMR-NIN (PCT)

| S.No | Name of the crop | Trans Gene/Protein   | Tests profile  | Remarks   |
|------|------------------|--|--|---|
| 1    | Brinjal          | <i>cry1Fa1</i> /Cry1Fa1 (event 142)  | <ul style="list-style-type: none"> <li>➤ Acute Toxicity test</li> <li>➤ Sub-Chronic Study (Leaves, Fruit )</li> <li>➤ Allergenicity studies :                             <ul style="list-style-type: none"> <li>▪ Pepsin Digestibility Assay,</li> <li>▪ Thermal Stability</li> </ul> </li> </ul>               | In market for 5- years<br>Bangladesh used this data |
| 2.   | Okhra            | <i>cry1Ac</i> /Cry1Ac  | ➤ Sub-Chronic Study (Fruit )   | RCGM approved                                       |
| 3.   | Cotton           | <i>cry1Ac</i> /Cry1Ac (event -1)<br>and<br><i>cry1EC</i> /Cry1EC (event -24) | <ul style="list-style-type: none"> <li>➤ Acute Toxicity test</li> <li>➤ Sub-Chronic Study (Leaves, Seeds )</li> <li>➤ Allergenicity studies :                             <ul style="list-style-type: none"> <li>▪ Bioinformatics Analysis of proteins,</li> </ul> </li> </ul>                                   |   |
| 4.   | Mustard          | Bar / Barstar / Barnase  | <ul style="list-style-type: none"> <li>➤ Acute Toxicity test</li> <li>➤ Sub-Chronic Study (Leaves, Seeds )</li> <li>➤ Compositional analysis</li> <li>➤ Allergenicity studies :                             <ul style="list-style-type: none"> <li>▪ Bioinformatics Analysis of proteins,</li> </ul> </li> </ul> | GAEC pending  |
| 5    | Rice             | <i>cry1Ab</i> /Cry1Ab  | ➤ Acute Toxicity test  | Sub-acute to be done                                |

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## How much should we consume?

The Expert Committee of the Indian Council of Medical Research, taking into consideration the nutrient requirements, has recommended that every individual should consume at least 300 g of vegetables (GLV : 50 g; Other vegetables : 200 g; Roots & Tubers : 50 g) in a day. In addition, fresh fruits (100 g), should be consumed regularly. Since requirements of iron and folic acid are higher for pregnant women they should consume 100g of leafy vegetables daily. High calorie vegetables and fruits to be restricted for over weight/ obese subjects.

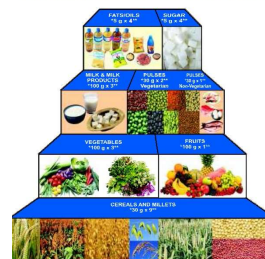


Figure 10  
BALANCED DIET FOR ADULT MAN (SEDENTARY)



\* Portion Size. \*\* No. of Portions

Figure 11  
BALANCED DIET FOR ADULT WOMAN (SEDENTARY)



\* Portion Size. \*\* No. of Portions

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## Exposure Levels

| S.no | Crop                                | Human Intake<br>gm/adult<br>(gm/kg) | Expression of protein (fresh) |                         | Rat/200g*<br>(gm/kg) |
|------|-------------------------------------|-------------------------------------|-------------------------------|-------------------------|----------------------|
|      |                                     |                                     | Per gm (100gm)                | Human intended exposure |                      |
| 1    | Bt.Brinjal (cry1Fa1 protein)        | 300<br>(5gm)                        | 4µg (400 µg)                  | 1.2mg                   | 5.4<br>(27.0)        |
| 2    | Bt.okra (Cry1Ac protein)            | 500<br>(8.3gm)                      | 35 µg (3500 µg)               | 17.5mg                  | 9<br>(45.0)          |
| 3    | GM Mustard( Bar, barnase & barstar) | 100<br>(1.7)                        | 94 µg (9400 µg)               | 9.4mg                   | 1.8<br>(9.0)         |
| 4    | Bt.Rice ( Cry1Ab(DG) protein)       | 600<br>(10)                         | 2.8 µg (280 µg)               | 1.7mg                   | 10.8<br>(54.0)       |

\* [Paget.G.E. & Barnes.J.M. (1964) Evaluation of Drug Activities: Pharmacometrics eds. Laurence.D.R & Bocharach.A.L, Vol.1. Academic Press, New York].

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## Example for exposure level

| S.no | Crop                         | Human Intake | Expression of protein (fresh) |                         | Animal<br>Dose(crop equiv)<br>gm/kg of rat* |
|------|------------------------------|--------------|-------------------------------|-------------------------|---|
|      |                              |              | Per gm (100gm)                | Human intended exposure |   |
| 1    | Bt.Brinjal (cry1Fa1 protein) | 300gm/Adult  | 4µg (400 µg)                  | 1.2mg                   | 27.0<br>gm/kg of rat*                       |

- \*1. Adult consumption: 300gm/day (Max. Daily Dietary Intake-NNMB)
- 2. Conversion factor (F): Human(60kg) to Rat(200gm)- 0.018
- 3. Rat(200gm)= 5.4gm (human consumption 300gm x F (0.018) =5.4 )
- 4. Human/kg -5 gm: Rat/kg- 27gm (5.6 times higher than the human diet)

Acute Exposure : 2gm/kg with pure(>60%) or equivalent protein

- 1. Protein used for Bt.Brinjal - cry1Fa1 protein – 200 times of protein expression (i.e 20mg/kg body weight of mice)
- 2. Rat Exposure : 40 mg/200 gm (33 times higher than intended human exposure)
- 3. Max. Human exposure/ day -1.2 mg

Human consume – minimum 0.1-1gm DNA / diet /day

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## INVESTIGATIONS FOR SAFETY PROFILE

| Test Details                            | Brief  |
|---|--|
| <b>1. Compositional analysis</b>        |  |
| Compositional analysis of key component | Compositional analysis of key components in transgenic Test material which are present in edible part.   |
| <b>2. Allergency studies</b>            |  |
| Bioinformatics Analysis of proteins     | Bioinformatic analysis of recombinant proteins expressed in transgenic Test material to assess potential allergenic-cross-reactivity to known allergens.             |
| Pepsin Digestibility Assay              | Assessment of Allergenicity of Recombinant Proteins Expressed in Transgenic Test material by <i>in vitro</i> Pepsin Digestibility Assay in Stimulated Gastric Fluid. |
| Thermal Stability                       | Assessment of Allergenicity of Recombinant Proteins Expressed in Transgenic Test material by Thermal Stability Assay <i>in vitro</i>                                 |

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## INVESTIGATIONS FOR SAFETY PROFILE contd...

| Study Details   | Duration                                   | Parameters   |
|---|--|--|
| <b>Acute Toxicity Study</b><br>single / multiple exposures within 24 hours (MTD)                                | <b>14 -D</b><br>Post Exposure Pure Protein | <ul style="list-style-type: none"> <li>✓ Cage side Observation (Daily)</li> <li>✓ Recording of body weights (Twice a week)</li> <li>✓ Physical Examination (Twice a week)</li> <li>✓ Neurological Examination (Twice a week)</li> <li>✓ Lethality (Daily)</li> </ul>   |
| <b>Sub-Chronic study</b><br>of test material for comparison of transgenic to their non-transgenic counterparts. | <b>90- D</b>                               | <ul style="list-style-type: none"> <li>✓ Cage side observation (Daily),</li> <li>✓ Physical Examination (Twice a week) Recording of body weights (Twice a week)</li> <li>✓ Recording of Feed intake (Daily)</li> <li>✓ Neurological Examination (Twice a week)</li> <li>✓ Urine analysis qualitative (Before &amp; after exposure to the test material)</li> <li>✓ Biochemistry, Hematology, Necropsy and Histopathology of vital organs (End of the euthanization)</li> </ul> |

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Example of study performance with **transgenic *Brassica juncea***  
Mustard

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**Pre clinical safety evaluation and Compositional analysis of key components in  
Leaves & Seeds of two events of transgenic *Brassica juncea***



**Study Centre**  
**Center For Advanced Research For Pre - Clinical Toxicology**  
**National Institute Of Nutrition (ICMR)**  
Hyderabad, INDIA, 500007  
Study No: 02/12



**Sponsor**  
**Centre for Genetic Manipulation of Crop Plants**  
**University of Delhi, South Campus**  
**Benito Juarez Road, New Delhi-110021**  
**Tel: 011-24115203**

**Study Approvals:** RCGM No. BT/BS/17/30/97-PID  
IBSC No. FDTRC/BIO-Safety/12  
IAEC No. P49/NCLAS/IAEC/2011/12/28

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20/30

NIN-PCT

## PRODUCT PROFILE & SOURCE

| S.no | Purified protein | Quantity received | Concentration(mg/ml) | Source  |
|------|------------------|-------------------|----------------------|---|
| 1    | Bar Protein      | 425mg             | 10                   | Supplied by<br>M/S Premas Biotech<br>Pvt. Ltd |
| 2    | Barnase Protein  | 408mg & 260mg     | 10                   |   |
| 3    | Barstar          | 722.5mg           | 17                   |   |

| S.no | Lyophilized powder & Fresh Leaf   | Quantity received | Seed   | Quantity received | Source  |
|------|---|-------------------|--|-------------------|---|
| 1    | Varuna (NT)            | 904g + 6kg        | Varuna (NT)            | 555g              | Navagaon,<br>Kumher & Sri<br>ganganagar<br>around 200<br>kms from<br>Delhi. |
| 2    | EH-2(NT)               | 770g + 6kg        | EH-2(NT)               | 555g              |   |
| 3    | Varuna<br>Barnase (T)  | 770g + 6kg        | Varuna<br>Barnase (T)  | 555g              |   |
| 4    | EH-2<br>Barstar (T)    | 750g + 6kg        | EH-2<br>Barstar (T)    | 555g              |   |
| 5    | DMH-11 (T)             | 792g + 6kg        | DMH-11(T)              | 555g              |   |

(NT- Non Transgenic),(T- Transgenic)

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## TEST DETAILS

| Volume                           | Test Details   | Objective  |
|----------------------------------|--|--|
| <b>1. Allergenicity studies</b>  |  |  |
| I                                | Bioinformatics Analysis of three proteins (Bar, Barnase and Barstar)   | Assessment of potential allergenic cross reactivity to known allergens   |
| II                               | Pepsin Digestibility Assay of the Bar, Barnase and Barstar proteins  | To assess digestibility of test proteins in pepsin in SGF at pH 1.2  |
| III                              | Thermal Stability of the Bar, Barnase and Barstar proteins   | To assess thermal stability of test proteins to varying temperatures and assess functional activity.   |
| <b>2. Acute Toxicity test</b>    |  |  |
| IV                               | Acute Oral Toxicity Of Bar Protein   | Safety Assessment of proteins at 1000mg/kg which is more than 10 folds higher than the intended expression levels  |
| V                                | Acute Oral Toxicity Of Barstar Protein   |  |
| VI                               | Acute Oral Toxicity Of Barnase Protein   |  |
| <b>3. Sub-Chronic Study</b>      |  |  |
| VII                              | Sub-chronic toxicity in Leaves from two events ( Varuna Barnase (VB)- Transgenic(T), Varuna - Non Transgenic (NT), EH2 Barstar (EH2B)-T, EH2 - NT, DMH-11 (Transgenic hybrid of VB & EH2B) | Safety Assessment of Leaves / Seeds of transgenic <i>Brassica juncea</i> lines compared to their non-transgenic counterparts in rats                       |
| VIII                             | Sub-chronic toxicity in Seeds from two events ( Varuna Barnase (VB)- Transgenic(T), Varuna - Non Transgenic (NT), EH2 Barstar (EH2B)-T, EH2 - NT, DMH-11 (Transgenic hybrid of VB & EH2B)  |  |
| <b>4. Compositional analysis</b> |  |  |
| IX                               | Compositional analysis of key component in Leaves, Seeds, of two events - Volume IX.   | Proximate Composition, Minerals Composition, Vitamins Composition, Secondary Metabolites and Phyto Sterols, Amino acid Composition, Fatty Acid Composition |

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## Assessment of Allergenicity by Bioinformatics Analysis

**Objective :** To identify potentially relevant matches in amino acid sequence between the Bar, Barnase and Barstar proteins and known or putative allergens.

**Methodology:** The databases PubMed, Allergen Online version 12.0 Allergen Database and NCBI Entrez Protein Database were used to accomplish the bioinformatics searches.

(<http://www.ncbi.nlm.nih.gov/sites/entrez>, <http://www.cbs.dtu.dk/services/SignalP/>, <http://www.cbs.dtu.dk/services/NetNGlyc/>, <http://www.allergenonline.org/>, [www.ncbi.nlm.nih.gov/protein](http://www.ncbi.nlm.nih.gov/protein))

### Results:

1. No significant amino acid identity matches between Bar, Barnase, and Barstar proteins with known allergens, with FASTA full length, 80 amino acid window, and 8 contiguous amino acid match search.
2. One very low level identity match of 35.7% identity over 84 amino acids found between Barnase and hen-egg lysozyme (Gal d 4), a minor egg white allergen. The alignment included 9 gaps, indicating poor sequence homology.

### Conclusion:

- ✓ No significant matches were identified to any allergenic protein.
- ✓ No scientific publications were identified on PubMed literature database that indicated any potential safety concerns.

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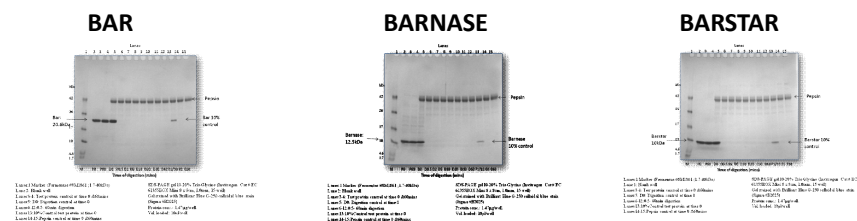
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## Assessment of Allergenicity by Pepsin Digestibility Assay

**Objective :** The allergenicity assessment of novel protein, intended for use in GM crops/foods, for stability in pepsin at acidic pH by pepsin digestion assay.

**Methodology:** Assess pepsin digestibility of the test proteins in SGF at pH 1.2, analyze digested proteins by SDS-PAGE and densitometry.

### Results:



**Conclusion:** The results of the pepsin digestibility assay showed that the Bar, Barnase and Barstar test proteins were rapidly degraded and 90% digestibility was achieved within 0.5 mins for all the three test proteins

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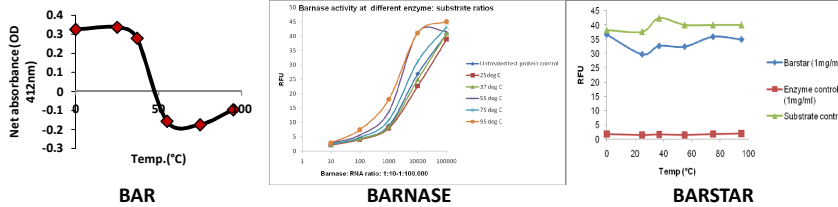
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## Assessment of Allergenicity by Thermal Stability Assay

**Objective :** The allergenicity assessment of purified recombinant protein (Bar, Barnase and Barstar) intended for use in GM crops/foods for thermal stability.

**Methodology:** The test protein were subjected to different temperatures (0, 25, 37, 55, 75 and 95°C for up to 30 minutes) followed by testing the enzyme activity.

**Results:**



**Conclusion:**

- The Bar recombinant protein showed a rapid decrease of activity from 130 units to -32.0 units/min/mg at temperatures from 55°C to 95°C.
- The results of the enzyme activity assay of Barnase (RNase) and Barstar (RNase inhibitor) recombinant proteins indicated that due to heat stability of these proteins, no change in the activity of these enzymes could be observed with heat treatment
- Allergy risk may be considered to be low/limited since Barnase and Barstar proteins are mainly expressed during the development stages of the plant in the anthers, are rapidly digested by pepsin in SGF and do not share any homology with known allergens

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## STUDY IMPRESSION – ALLERGENICITY STUDIES

### Bioinformatics

- No significant matches were identified to any allergenic protein.

### Pepsin digestibility

- Bar, Barnase and Barstar test proteins were rapidly degraded which suggest no allergy risk.

### Thermal Stability

- No Allergy risk except low / limited with Barnase and Barstar proteins

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## Acute Toxicity study of recombinant Bar, Barstar and Barnase proteins (14days)

### Study Design : SA Mice, 4 –6 weeks & 18 – 20 gms

| S.no | Test compound            | No. of animals | Dosage schedule (oral route) |                        | Study parameters       |
|------|--------------------------|----------------|------------------------------|------------------------|------------------------|
|      |                          |                | mg/2ml                       | Duration               |                        |
| 1    | Vehicle control (buffer) | 16 (8 ♂ + 8 ♀) | Solubilized protein buffer   | 0.5 ml x 4 times a day | Activity and lethality |
| 2    | Bar protein              | 16 (8 ♂ + 8 ♀) | 20mg*                        |                        |                        |
| 3    | Barstar protein          | 16 (8 ♂ + 8 ♀) | 34 mg*                       |                        |                        |
| 4    | Barnase protein          | 12 (6 ♂ + 6 ♀) | 20mg*                        |                        |                        |

10 times > the expression levels, Excluding the conditioning period of 7–10 days.  
Maximum of 2 ml in 24 hrs (0.5 ml every 6 hours).

Rationale : Mice - Oral exposure with - 1000mg/kg of Bar, Barnase & Barstar proteins<sup>1</sup>

①

Bar - 800 times more than the expression level in leaves (94µg/g)

Barnase - 800 times more than the expression level in leaves

Barstar - 1360 times more than the expression level in leaves

? Expression levels

### Results

- ✓ Pre-terminal mortality<sup>@</sup> was recorded in control group (Female Mouse – 1/14<sup>th</sup> day).
- ✓ No mortality in animals exposed to test protein.
- ✓ Gain in body weight, food intake was normal.

@ Pulmonary pathology with pneumonic changes

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## Sub chronic Toxicity Study in Rats

### Rationale:

| Consumption                           | Leaf/day  | Seed/day        |
|---------------------------------------|---|-----------------|
| Human DDI <sup>1</sup>                | 100gm   | 0.53gm          |
| Rat <sup>2</sup>                      | 100 X 0.018 = fresh weight - 1.8gm  | 10mg (0.0095gm) |
| Wet Weight of Test material (WWT)     | 1.8gm   | -----           |
| Lyophilized Powder (LP) of WWT*       | 0.27gm (without 85% moisture)   | -----           |
| Actual fed (powdered) <sup>@</sup>    | 0.4gm   | 20mg            |
| Bar protein expression <sup>5,3</sup> | 94µg/gm   | -----           |
| Total Bar Protein consumption         | 0.244mg in 0.4gm of LP * 1.4 times > intended human consumption (0.14 / 0.24) | -----           |

\* Lyophilized powder prepared by reduction of 85% moisture with Fresh leaves weight

@ To overcome the losses Test material fed to Rat is 0.4gm lyophilized leaf and 20mg of seed

<sup>5</sup> Maximum expression levels as per the 94µg/gm, ratio of 1000 to 1000000

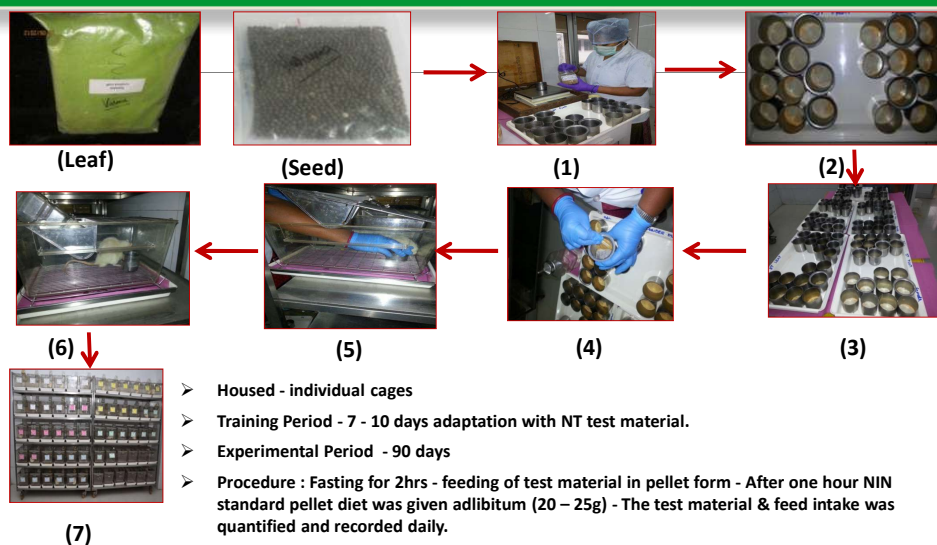
Rat's physiology - similar to human being; one-day-old rat is similar to a six-month-old baby. Mandatory to generate the pre-clinical toxicity data in rodent species.  
Dietary profile – similar to human DDI.

1. Dietary guidelines for Indians, A Manual, NIN, Hyderabad. (Page no-42)
2. Conversion profile (conversion factor of rat-0.018) [Paget.G.E. & Barnes.J.M. (1964) Evaluation of Drug Activities: Pharmacometrics Ed. Laurence.D.R & Bocharach.A.L., Vol.1. Academic Press, New York].
3. Sponsors Information.

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## Test Material Feeding Procedure



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## Sub-chronic toxicity study - Leaf

Study Design : SD rats, 6 –8 weeks & 170 – 200 gms

Maximum Dietary intake: 100g / adult

| S.No | Group Details  | No. of animals /sex | Dietary intake g/rat <sup>#</sup> | Study Period <sup>®</sup>                  | Study parameters   |
|------|----------------|---------------------|-----------------------------------|--|--|
| 1    | Control        | 20(10♂+10♀)         | NIN normal diet (NP)              | 114 days<br>(Duration of Exposure-90 days) | Cage side observation (daily), Physical examination (twice a week), Recording of body weights, Neurological examination, Urine analysis qualitative, Biochemistry, Hematology, Necropsy and Histopathology of vital organs. Immunology: tier i & tier ii tests |
| 2    | Varuna (NT)    | 20 (10♂+10♀)        | 0.4 + NP                          |  |  |
| 3    | EH2 (NT)       | 20 (10♂+10♀)        | 0.4 + NP                          |  |  |
| 4    | Varuna Barnase | 20 (10♂+10♀)        | 0.4 + NP                          |  |  |
| 5    | EH2 Barstar    | 20 (10♂+10♀)        | 0.4 + NP                          |  |  |
| 6    | DMH-11         | 20 (10♂+10♀)        | 0.4 + NP                          |  |  |

<sup>#</sup>: Maximum amount of Test material administered is 0.4g/200g (2g/ kg B.Wt./day).

NP: 1.1g/rat standard NIN powder diet was mixed

NT – Non-transgenic; NP - Standard NIN powder diet

♂=Male; ♀=Female

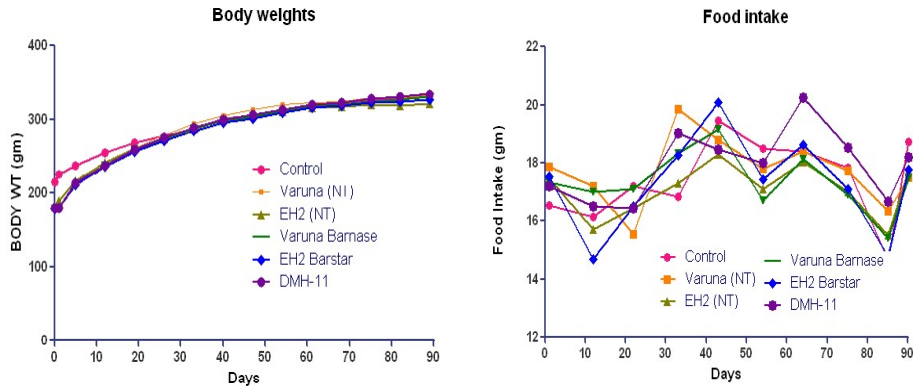
<sup>®</sup> : With 7 days Acclimatization + 3 days pre urine examination + 10 days Adaptation to Diet Regime + 90days exposure +2 days post urine examination +2 days for euthanization.

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## RESULTS

### Sub chronic toxicity study of leaves from two events of Transgenic *B. juncea* Sex Pooled (Rats)



No significant difference in body weights, feed intake, and cage side activities.

Values are expressed as Mean ± Standard Deviation

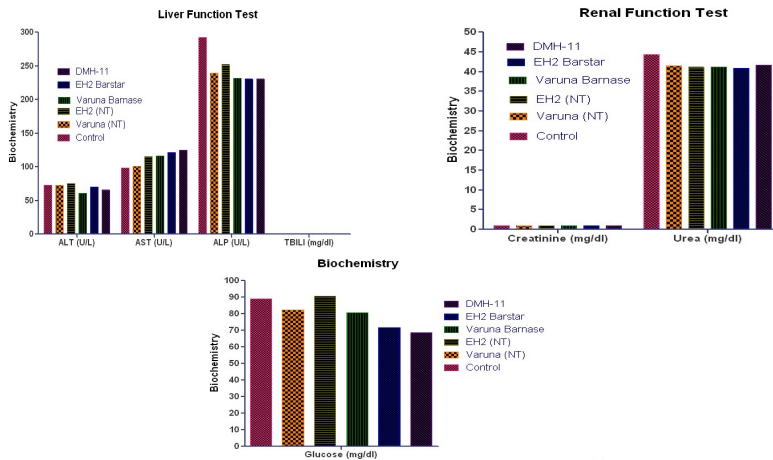
( ) No. of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of leaves from two events of Transgenic *B. juncea* BIOCHEMICAL PARAMETERS – Sex Pooled (Rats)



Values are expressed as Mean ± Standard Deviation

( ) No. of animals

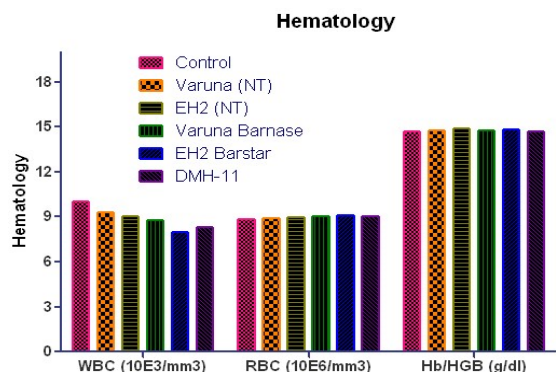
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## RESULTS (Contd....)

### Sub chronic toxicity study of leaves from two events of Transgenic *B.juncea* HEMATOLOGY - Sex Pooled (Rats)



Hematology profile was within normal range.

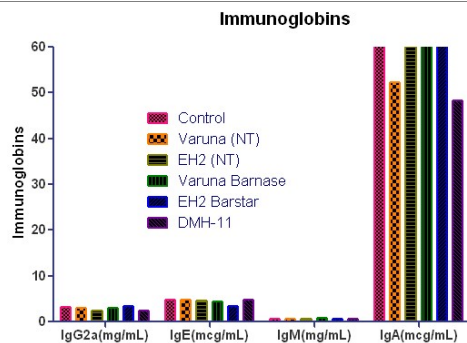
Values are expressed as Mean  $\pm$  Standard Deviation ( ) No of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of leaves from two events of Transgenic *B.juncea* IMMUNOLOGICAL PARAMETERS - Sex Pooled (Rats)



- Serum total IgG2a, IgE, IgM and IgA levels were not altered in animals fed with transgenic material and were comparable with those fed with non-transgenic material.
- No allergenicity symptoms seen in the animals.

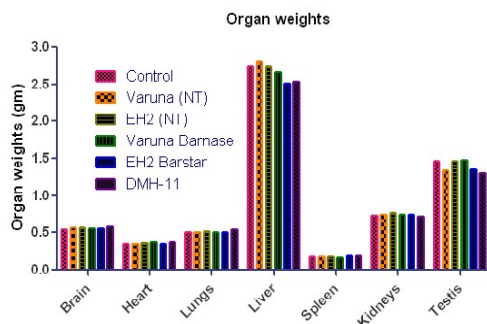
Values are expressed as Mean  $\pm$  Standard Deviation ( ) No. of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of leaves from two events of Transgenic *B. juncea* Organ weights - Sex Pooled (Rats)



- No mortality in any group of animals which received transgenic or non-transgenic leaves of *B. juncea* at the dose of 0.4g/rat/day for 90 consecutive days.
- Histopathology evaluation was also unremarkable.

Values are expressed as Mean ± Standard Deviation ( ) No of animals

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October 9-10, 35/30  
NIN-PCT

## Sub-chronic toxicity study - Seed

Study Design : SD rats, 6 –8 weeks & 170 – 200 gms

Maximum Dietary intake (Human/adult): 0.53g / day

| S.No | Group Details  | No. of animals /sex | Dietary intake mg/ rat/day <sup>#</sup> | Study Period <sup>+</sup>                 | Study parameters  |
|------|----------------|---------------------|---|---|---|
| 1    | Control        | 20 (10♂+10♀)        | NIN normal diet (ND)                    | 119days<br>(Duration of Exposure-90 days) | Cage side observation (daily),<br>Physical examination (twice a week),<br>Recording of body weights,<br>Neurological examination,Urine<br>analysis qualitative, Biochemistry,<br>Hematology, Necropsy and<br>Histopathology of vital rgans.<br>Immunology: tier i & tier ii tests |
| 2    | Varuna (NT)    | 20 (10♂+10♀)        | 20 + NP                                 |   |   |
| 3    | EH-2 (NT)      | 20 (10♂+10♀)        | 20 + NP                                 |   |   |
| 4    | Varuna Barnase | 20 (10♂+10♀)        | 20 + NP                                 |   |   |
| 5    | EH-2 Barstar   | 20 (10♂+10♀)        | 20 + NP                                 |   |   |
| 6    | DMH-11         | 20 (10♂+10♀)        | 20 + NP                                 |   |   |

<sup>#</sup>: Maximum amount of Test material administered is 20mg/200g (100mg/ kgB.Wt./day).  
<sup>NP</sup>: 980mg/rat standard NIN powder diet was mixed  
 NT – Non-transgenic, ND - Normal diet  
 ♂=Male; ♀=Female  
<sup>+</sup>: With 15 days Acclimatization + 2 days pre urine examination + 9 days Adaptation to Diet Regime + 90days exposure + 1 day pre urine examination and 2 days for euthanization.

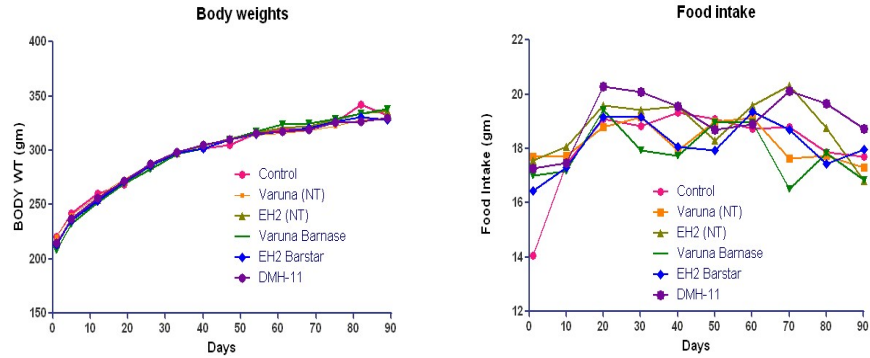
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## RESULTS

### Sub chronic toxicity study of seeds from two events of Transgenic *B.juncea*

Sex **Pooled** (Rats)



No abnormal findings with reference to gain in body weight, feed intake, cage side activity and clinical observations.

Values are expressed as Mean ± Standard Deviation

( ) No of animals

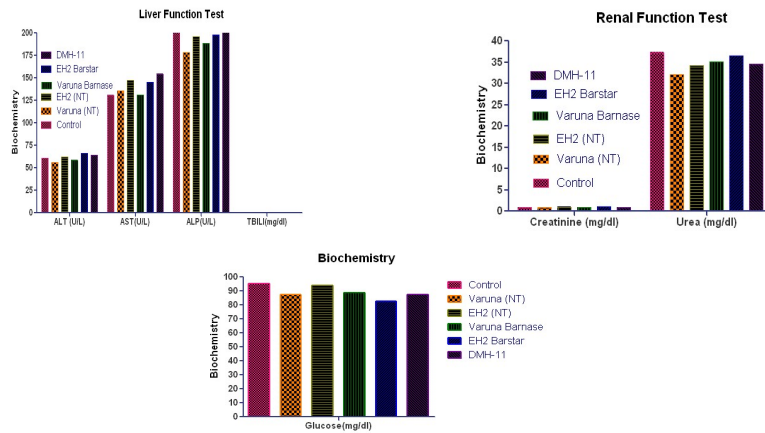
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## RESULTS (Contd....)

### Sub chronic toxicity study of seeds from two events of Transgenic *B.juncea*

BIOCHEMICAL PARAMETERS - Sex **Pooled** (Rats)



Values are expressed as Mean ± Standard Deviation

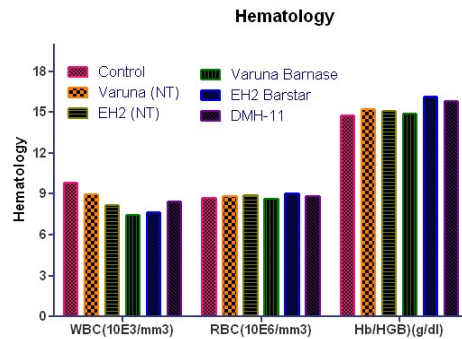
( ) No of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of seeds from two events of Transgenic *B. juncea* HEMATOLOGY - Sex Pooled (Rats)



Hematological observations were within normal range.

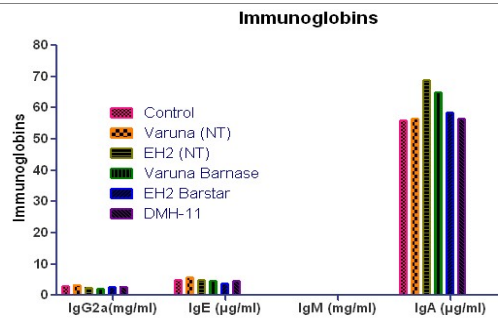
Values are expressed as Mean  $\pm$  Standard Deviation ( ) No of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of seeds from two events of Transgenic *B. juncea* IMMUNOLOGICAL PARAMETERS - Sex Pooled (Rats)



- Serum total IgG2a, IgE, IgM and IgA levels were not altered with transgenic and were compared with non-transgenic *B. juncea* seed fed animals.
- No allergy symptoms seen in the test and control animals.

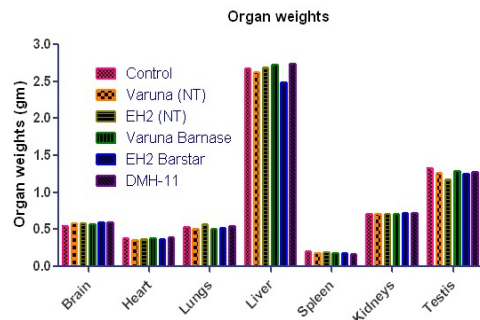
Values are expressed as Mean  $\pm$  Standard Deviation ( ) No of animals

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## RESULTS (Contd....)

### Sub chronic toxicity study of seeds from two events of Transgenic *B. juncea* Organ weights - Sex Pooled (Rats)



- No pre-terminal deaths were observed in any group of animals in sub-chronic toxicity test which received test material.
- The histopathological result was also seen to be unremarkable.

Values are expressed as Mean  $\pm$  Standard Deviation

( ) No of animals

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## Study impression :

Leaf: The Daily Dietary Intake (DDI) of *B. juncea* (transgenic & non transgenic) leaves (0.4 g/day) for 90days by rats suggest safety profile as assessed by the following parameters\*. DDI  $\equiv$  100gm human intake

Seed : The Daily Dietary Intake (DDI) of *B. juncea* (transgenic & non transgenic) seeds (20mg/day) for 90days by rats suggest safety profile as assessed by the following parameters\*. DDI  $\equiv$  0.53gm human intake

- \* Physical examination (twice a week),  
Recording of body weights,  
Neurological examination,  
Urine analysis qualitative, Biochemistry,  
Hematology,  
Necropsy and Histopathology of vital organs.  
Immunology: tier i & tier ii tests



Leaf



Seed

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## Compositional Analysis of *Brassica juncea* Leaf And Seed Methodology

- The Leaf samples of each transgenic (3), non –transgenic (2) and Zonal check
- Randomly collected from three different places within each area\*.
- Homogenization of samples to prepare 1kg aliquot in banana leaves .
- Transported to Study Centre by Airlines in Thermacol box.

Navagaon

Kumher

Sri Ganganagar

| Composition                            | Composition Profile  | Method            |
|--|--|-------------------|
| Proximates                             | Crude Protein  | AOAC 984.13       |
|  | Crude Fat  | AOAC 2003.06      |
|  | Ash  | AOAC 942.05       |
|  | Carbohydrate   | AOAC 986.05       |
|  | Sugars, Total  | AOAC 974.06       |
| Fibre                                  | Acid detergent Fibre   | AOAC 973.18       |
|  | Neutral detergent Fibre  | AOAC 2002.04      |
|  | Total Dietary Fibre  | AOAC 985.29       |
| Secondary Metabolites and Phytosterols | Phytic acid  | AOAC 986.11       |
|  | Sinapine   | Internal Method-2 |
|  | Cholesterol, Brassicasterol, Campesterol, Stigmasterol, Beta sitosterol, Total sterols | AOAC 994.10       |
|  |  |                   |

\* Centre space of lamina

Outer space of lamina

Different sizes of leaves

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## Compositional Analysis of *Brassica juncea* Leaf And Seed (Contd....)

| Composition             | Composition Profile  | Method   |              |
|-------------------------|--|--|--------------|
| Micro & Macro Nutrients | Ca (Calcium)   | AOAC 985.01                                      |              |
|                         | Fe (Iron)  |  |              |
|                         | Mg (Magnesium)   |  |              |
|                         | P (Phosphorous)  |  |              |
|                         | K (Potassium)  |  |              |
|                         | Na (Sodium)  |  |              |
|                         | Zn (Zinc)  |  |              |
|                         | Cu (Copper)  |  |              |
|                         | Mn (Manganese)   |  |              |
|                         | Se (Selenium)  | Internal Method-1                                |              |
|                         | B1 (Thiamine)  |  |              |
|                         | B2 (Ribofavin)   |  |              |
|                         | B3 (Niacin)  |  |              |
|                         | B5 (Pantothenic acid)  |  |              |
|                         | Vitamins   | B6 (Pyridoxine)                                  | AOAC 2005.07 |
|                         |  | B9 (Folic Acid)                                  |              |
|                         |  | Lutein   |              |
|                         |  | Beta-carotene                                    |              |
|                         |  | Vitamin E (alpha-tocopherol)                     |              |
| Amino Acids             | Vitamin K (Phylloquinone)  | AOAC 999.15                                      |              |
|                         | Vitamin C (Ascorbic acid)  | AOAC 985.33                                      |              |
|                         | Asp, Thr, Ser, Glu, Pro, Gly, Ala, Cys, Val, met, Ile, Leu, Tyr, Phe, His, Lys, Art, Trp | Waters AMQ derivatization                        |              |
|                         | Fatty acids composition  | Saturated, Unsaturated (Mon and Poly), Trans fat | AOAC 996.06  |

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## Study Impression:

| Composition                            | Composition profile                  | STATISTICAL SINGIFICANCE |     |
|--|--------------------------------------|--------------------------|-----|
|  |                                      | VARUNA                   | EH2 |
| Proximate &Fibre                       | Total Dietary Fibre(Leaf Dry)        | +                        | -   |
|  | Total Dietary Fibre(Seed Dry)        | -                        | +   |
| Minerals                               | Magnesium(Seed Fresh)                | -                        | +   |
| Vitamins                               | Leutin(Leaf Dry& Fresh)              | +                        | -   |
|  | Vitamin-C(Leaf Dry& Fresh)           | +                        | +   |
|  | Vitamin-E (Seed dry)                 | -                        | +   |
| Secondary Metabolites and Phytosterols | Cholestrol(Leaf&Seed)                | -                        | +   |
| Amino Acids                            | Leucin(Leaf Fresh)                   | +                        | -   |
| Fatty Acids (complete profile)         | C18:2n6c Linolenic (Leaf Dry& Fresh) | +                        | -   |

- These changes are expected due to agro-climatic /demographic changes
- The above changes indicate substantial equivalence in composition between transgenic and non transgenic Brassica.

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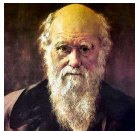
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## GM crops: Time to be responsible & responsive

- Opposition to GM crops in India, like elsewhere.
- The Technical Expert Committee (TEC) appointed by the Supreme Court emphasized on a science-based cautious approach towards open releases of genetically-modified organisms (GMOs).
- It proves that the science community continues to have concerns around GM crops.

THE ECONOMIC TIMES 25 JAN, 2013, 06.01AM IST,

Repetition this time too "people always oppose the new inventions and findings"



Charles Darwin

- During Nehruji's time INDIA faced severe opposition against Hybrid Seeds. ? GREEN REVOLUTION
- Galileo was hacked for his finding in the solar system at ancient time (400 years back)
- Charles Darwin was shown the way out of Church for his Theory of Natural Selection (200 years)



Gallelio

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46

## 9 Reasons why An Apple a Day Really Keeps the Doctor away ?

1-Apple contains Vitamin C, 2-Prevent Heart Diseases,  
3-Low in calories, 4-Prevent Cancers, 5-Apples contain phenols,  
6-Prevent tooth decay, 7-Protects your brain from brain disease, 8-Healthier Lungs, 9-They taste great!



MYTH BUSTED



An Apple a day brings dentist closer ?

Eating regularly 4 times damages 2mm surface enamel of teeth

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## FACTS AND EXPERINCE

- Human consume – Minimum 0.1-1gm DNA / Diet /day
- Transgenic corn - 0.0001% / Total DNA
- Meta analysis – 147 original studies (soya, maize, cotton).
- Reduced pesticides by 37%, increased 22%, profits 68%.
- Multi generation studies (52 week feeding of soya 2007, 12 multi generation upto 5 generation) - No ' Evidence of Health Hazard'.
- Codex , WHO , European , US-FDA - Uniform approach.
- Europe consumption of GM is existing – but cultivation is restricted



Sterling Report - Seralini- 2012 Food Chem.Toxic. 4221-31).  
Statistical Fishing trips – with 10 rats v/s 65 or more and 24 months ?

- No proper controls
- Aged Animals
- No proper species

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## RAT'S AGE VERSUS HUMAN'S AGE: WHAT IS THE RELATIONSHIP?

ABCD Arq Bras Cir Dig Review Article 2012;25(1):

| Rat's age in months | Human's age in years |
|---------------------|----------------------|
| 6 months            | 18 years             |
| 12 months           | 30 years             |
| 18 months           | 45 years             |
| 24 months           | 60 years             |
| 30 months           | 75 years             |
| 36 months           | 90 years             |
| 42 months           | 105 years            |
| 45 months           | 113 years            |
| 48 months           | 120 years            |

|                      |               |                |
|----------------------|---------------|----------------|
| Total lifespan:      | 13.8 rat days | = 1 human year |
| Nursing Period:      | 42.4 rat days |                |
| Prepubescent Period: | 4.3 rat days  |                |
| Adolescent Period:   | 10.5 rat days |                |
| Adult Phase:         | 11.8 rat days |                |
| Aged Phase:          | 17.1 rat days |                |
| Average:             | 16.7 rat days |                |

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? Preclinical predictors of Clinical Safety

OR

Opportunities For Improvement

150-COMPOUNDS DATA PREDICTS

RODENT TOXICOLOGY – 43%- HUMAN TOXICITIES  
NON-RODENT TOXICOLOGY – 63%- HUMAN TOXICITIES  
TOGETHER – 71%- HUMAN TOXICITIES

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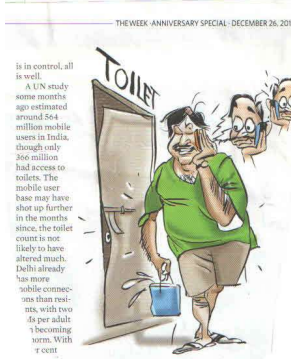
# WHICH IS FIRST & WHICH IS NEXT

## ETHICS For Whom ?

### SAFETY

- Animal studies
- Environmental
- Post marketing reports

**FIRST TEXT, TOILET NEXT**  
There is more mobile access than toilets in rural and urban areas



### EFFICACY

*Bt* cotton in India  
Maize, Soya  
mustard, Brinjal  
many in other  
countries

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## Evolution is the unifying force in modern biology ....

But Biotechnology remains a source of misunderstanding and controversy.  
Start finding out why it is so important with our beginner's guide

### Domestication of corn

#### Teosinte



9000  
years ago?

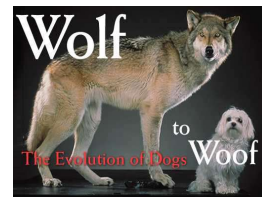
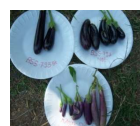


#### Hybrid



Bio-tech

#### Bt- Indian



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## CAN WE FACE CHALLENGES ?

### Bt Brinjal is safe, claims NIN However, Activists Against GM Crops Need To Be Convinced

**Hyderabad:** Is Bt Brinjal safe? The deliberations of Bt crops got a push with the parliament's committee on environment in its report submitted last week, commenting that transgenic Bt food crops would be brought with confidence to the Indian market. The report also urged the National Institute of Advanced Agricultural Sciences (NIAS) of the Indian Council of Agricultural Research (ICAR) to ensure that Bt Brinjal is safe.

A submission report on the laboratory experiments carried out on the safety of Bt Brinjal was submitted to the Review Committee on Genetic Manipulation (RCGM) of the Department of Biotechnology, Ministry of Science and Technology.

It is worth noting that the Bt Brinjal and Bt Brinjal Technology Research Centre, at the NIAS, had filed a court case against the Bt Brinjal was found to be safe. "What new needs to be done is open field trials," said M. K. Murali.

However, the problem arises here, in



and farmers opposed a transgenic Bt Brinjal. In the absence of scientific consensus and approval from state agencies such as APJCC, the Ministry has been imposed to put its trust on the commercial use of Bt Brinjal until government approval.

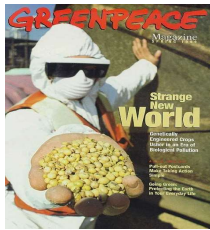
Activists also actively opposed the introduction of Bt Brinjal in N. Andhra Pradesh, founder director of the Centre for Cell Culture and Molecular Biology, Hyderabad, who was interviewed by the Hyderabad Times.

Those who had been opposed against Bt Brinjal said the government's committee on genetic manipulation only after the report was submitted.

The panel also said that the NIAS should ensure that the Bt Brinjal is safe to be used in the market. However, on February 20, 2010, the ministry of environment



**YES!**  
LET US PREPARE IDENTIFY THE INTERVENTION STRATEGIES



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## What all we have to Ban ?



Shall we stop ?



ONLY OPTION



SNAIL MAIL ZOOMING BACK.

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## THE UNBEATABLES

HEALTH CANNOT BE BUILT AT  
PHARMACY COUNTER



ECONOMICAL, FRESH, TASTY,  
BETTER ABSORBED AND RETAINED.  
AVAILABLE IN NATURAL FORM AND  
RICH IN FIBER.

EXPENSIVE, SYNTHETIC, HAZARDOUS  
IF TAKEN IMPROPERLY / IN EXCESS.  
PRODUCE UNWANTED SIDE EFFECTS.

Concept (1994); Dinesh Kumar & Kamala Krishna Syam