"CASE STUDY
PREMARKET SAFETY ASSESSMENT - GM MUSTURD"

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DRUG TOXICOLOGY RESEARCH CENTER

PATHOLOGY

NATIONAL CENTRE FOR LABORATORY ANIMAL SCIENCES

IMMUNOLOGY

STATISTICS

PCT Team
Pharmacologist
Pathologist
Veterinary Pathologist
Biochemist
Trained Technical Staff

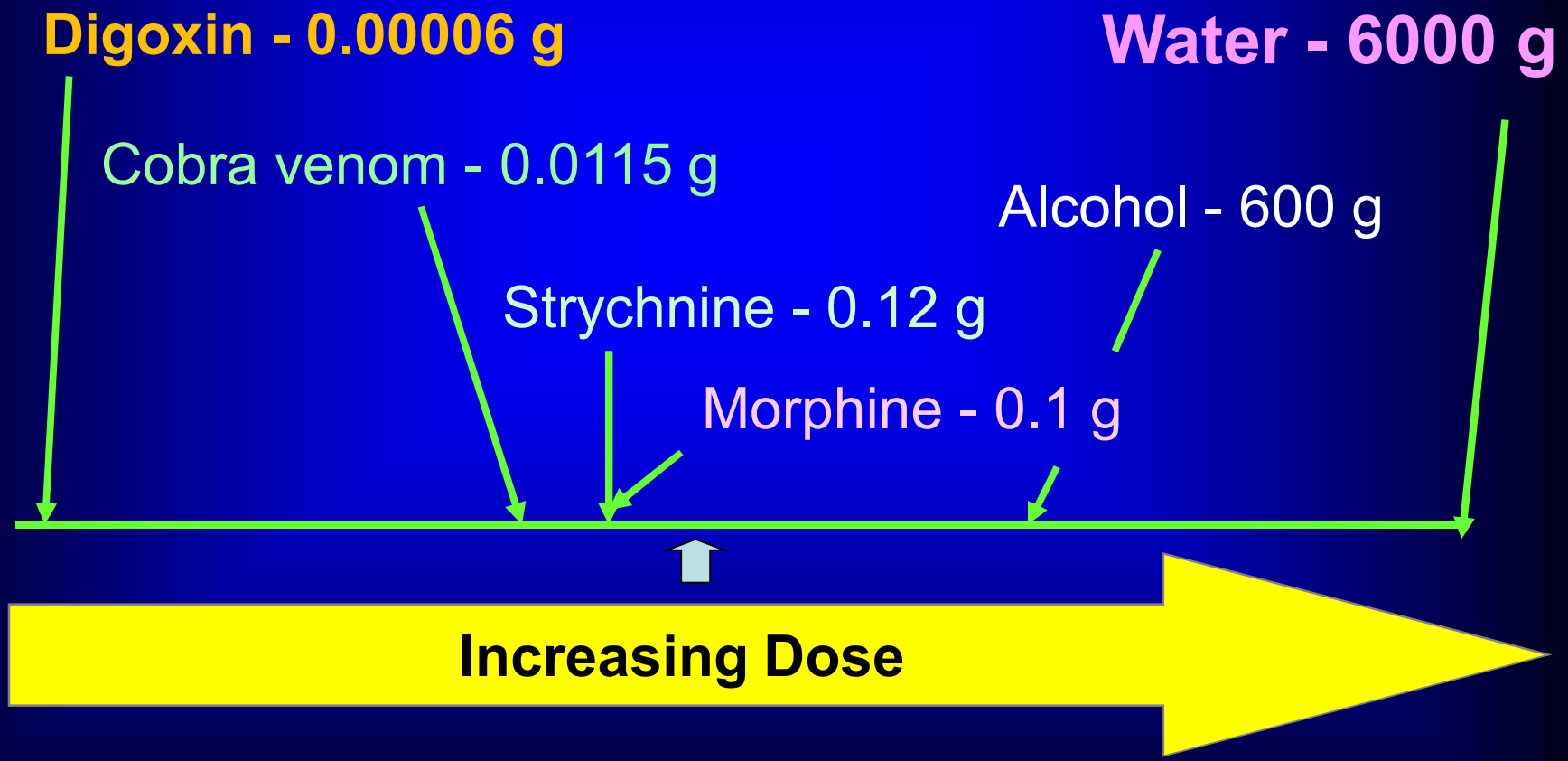
PCT Team
Geneticist
Microbiologist
Animal Scientists
Statistician
Trained Technical Staff

Team Work

CAN SAFETY BE PROVEN?

- It is impossible to prove that something is safe
- The best that can be demonstrated is the absence of evidence of the production of harm
- Present and future safety can only be judged on the basis of past experience, an absence of evidence of harm is the only evidence we can ever expect for the absence of harm.

"ALL THINGS ARE TOXIC, IT IS ONLY A MATTER OF EXPOSURE LEVEL / DOSE"



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 Director	**: Dr. B. Dinesh Kumar**
Study Investigators	**: Dr. P. Uday Kumar, Dr. R. Hemalatha Dr. S. Vasanthi, Dr.N.Hari Shankar, Dr. M.V. Surekha**
Collaborators	**: Prof. Deepak Pental, Dr. Vibha Gupta,**
Study Coordinators	**: DR. B. Sesikeran (till 2012), Dr. Vibha Ahuja**

Study Approvals:

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

GENETICALLY MODIFIEDCROPS - SCREENED at PCT NIN

S.No	Name of the crop	Trans Gene/Protein	Expression in edible portion
1	Bt.Brinjal 	*cry1Fa1* /Cry1Fa1 (event 142)	0.0004gm/% 4µg (400 µg)
2	GM Mustard 	*bar*/Bar	0.0094gm/% 94 µg (9400 µg)
		barstar / Barstar	X
		barnase/Barnase	X
3	Bt. Okra 	*cry1Ac*/Cry1Ac	0.0035gm/% 35 µg (3500 µg)
4	Bt. Cotton 	*cry1Ac* /Cry1Ac (event -1)	0.00040/% 40.47µg (4047µg)
		cry1EC /Cry1EC (event -24)	0.0015/% 15.2µg (1520µg)
5	Bt. Rice 	*cry1Ab*/Cry1Ab	0.00028gm/% 2.8 µg (280 µg)

TEST DETAILS (Brassica)

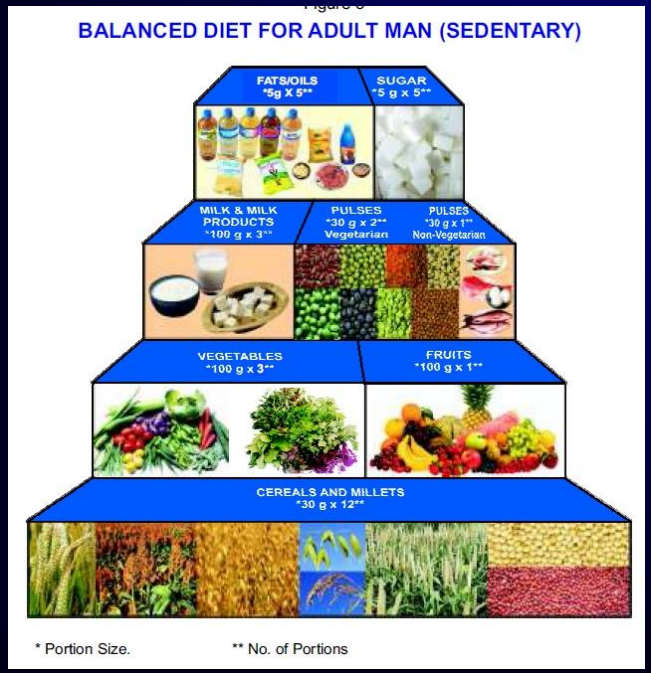
Volume	Test Details	Objective
1. Allergencity studies		
I	Bioinformatics Analysis of three proteins (Bar, Barnase and Barstar)	Assessment of potential allergenic cross reactivity to known allergens
II	Pepsin Digestibility Assay of the Bar, Barnase and Barstar proteins	To assess digestibility of test 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.
2. Acute Toxicity test		
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	
3. Sub-Chronic Study		
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 *Brassica juncea* 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)	
4. Compositional analysis		
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

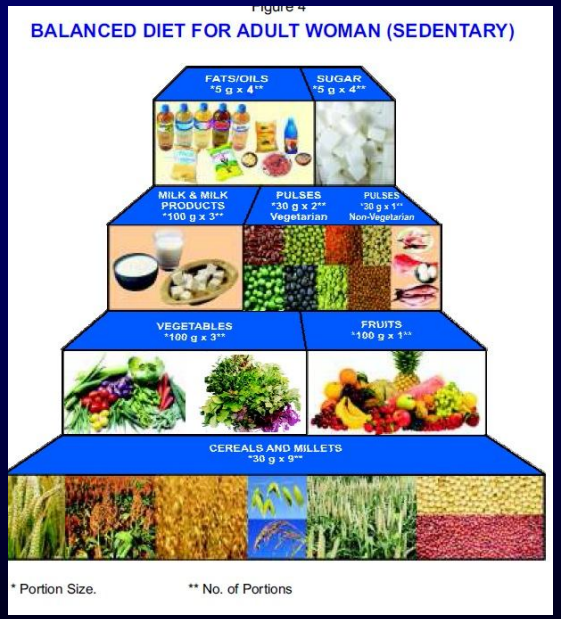
STUDY RATIONALE

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.

Source:
Dietary guidelines for Indians Published by NIN based on data of National Nutrition Monitoring Bureau (NNMB)

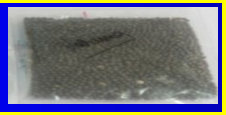
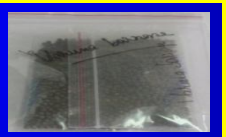
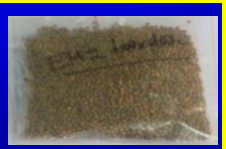




METHODOLOGY

PRODUCT PROFILE & SOURCE

S.no	Purified protein	Quantity received	Concentration(mg/ml)	Source
1	Bar Protein	425mg	10	Supplied by M/S Premas Biotech 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, Kumher & Sri ganganagar* around 200 kms from Delhi.
2	EH-2(NT) 	770g + 6kg	EH-2(NT) 	555g	
3	Varuna Barnase (T) 	770g + 6kg	Varuna Barnase (T) 	555g	
4	EH-2 Barstar (T) 	750g + 6kg	EH-2 Barstar (T) 	555g	
5	DMH-11 (T) 	792g + 6kg	DMH-11(T) 	555g	

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

Compositional Analysis of GM Food Crops

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

* Centre space of lamina
Outer space of lamina
Different sizes of leaves

Compositional Analysis

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

Compositional Analysis of GM Food Crops

Composition		Composition Profile	Method
Micro & Macro Nutrients	Minerals	Ca (Calcium)	AOAC 985.01
		Fe (Iron)	
		Mg (Magnesium)	
		P (Phosphorous)	
		K (Potassium)	
		Na (Sodium)	
		Zn (Zinc)	
		Cu (Copper)	
		Mn (Manganese)	
		Se (Selenium)	
	Vitamins	B1 (Thiamine)	Internal Method-1
		B2 (Ribofavin)	
		B3 (Niacin)	
		B5 (Pantothenic acid)	
		B6 (Pyridoxine)	
		B9 (Folic Acid)	
		Lutein	AOAC 2005.07
		Beta-carotene	
		Vitamin E (alpha-tocopherol)	AOAC 2012.09
		Vitamin K (Phylloquinone)	AOAC 999.15
		Vitamin C (Ascorbic acid)	AOAC 985.33
	Amino Acids	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

STUDY RATIONALE & EXPOSURE LEVEL

GM mustard - three proteins

Bar - Expression level in leaves - 94µg/g.
Barnase - Expression level in leaves
Barstar - Expression level in leaves

? Expression levels

S.no	Crop	Human Intake (Rat intake)	Expression of protein (fresh)			
			Per gm (100gm)	intended exposure		
				Human	Rat	Mice
1	GM Mustard(Bar, barnase & barstar)	100 g /Adult (1.8gm/200g rat)	94 µg (9400 µg)	9.4mg	0.17mg	0.02mg

1. Adult consumption: 100gm/day (Max. Daily Dietary Intake-NNMB)
2. Conversion factor (F): Human(60kg) to Rat(200gm)- 0.018
3. Rat(200gm)= 1.8gm (human consumption 100gmx F (0.018) =1.8)
4. Conversion factor (F): Human(60kg) to Mice(20gm)- 0.0026
5. Mice (20gm)=0.26gm (human consumption 100gmx F (0.0026) = 0.26)

Rationale : Mice - Oral exposure with - 1000mg/kg of Bar, Barnase & 1700mg/kg of Barstar proteins

Acute Toxicity study (14days)

Recombinant Bar, Barstar and Barnase proteins

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*		
Maximum of 2 ml in 24 hrs (0.5 ml every 6 hours).					

Rationale :

1. **Bar protein expression level is 94µg/gm**
2. **Human consumption = 100gm/60kg man (9.4mg Bar protein /100gm of leaves)**
3. **Maximum Bar & Barnase protein administered was 1000mg/kg based on the maximum solubility that could be achieved.**
4. **Maximum Barstar protein administered was 1700mg/kg based on the maximum solubility that could be achieved.**

Sub chronic Toxicity Study in Rats

Rationale:

Consumption	Leaf/day	Seed/day
Human DDI[1]	100gm	0.53gm
Rat[2]	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)@	0.4gm	20mg
Bar protein expression$,3	94µg/gm	---------
Total Bar Protein consumption	0.17mg in 0.4gm of LP	---------
* 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		
$ Maximum expression levels as per the 94µg/gm,		

Rationale for Animal Selection:

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.

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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: Pharmocometrics Ed. Laurence.D.R & Bocharach.A.L., Vol.1. Academic Press, New York].
3. Sponsors Information.

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#	Study Period@	Study parameters
1	Control	20(10♂+10♀)	NIN normal diet (NP)	114 days (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		

#: 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

@ : 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.

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#	Study Period+	Study parameters
1	Control	20 (10♂+10♀)	NIN normal diet (ND)	119days (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 rgans. 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		

#: Maximum amount of Test material administered is 20mg/200g (100mg/ kgB.Wt./day).
NP: 980mg/rat standard NIN powder diet was mixed
NT – Non–transgenic, ND - Normal diet
♂=Male; ♀=Female
+: 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.

OBSERVATION DETAILS

Study Details	No. of animals	Parameters
Acute Toxicity study	All	✓ Cage side Observation (Daily) ✓ Recording of body weights (Twice a week) ✓ Physical Examination (Twice a week) ✓ Neurological Examination (Twice a week) ✓ Lethality (Daily)
Sub-Chronic study	All	✓ Cage side observation (Daily), ✓ Physical Examination (Twice a week) Recording of body weights (Twice a week) ✓ Recording of Feed intake (Daily) ✓ Neurological Examination (Twice a week) ✓ Urine analysis qualitative (Before & after exposure to the test material) ✓ Biochemistry, Hematology, Necropsy and Histopathology of vital organs (End of the euthanization)

RESULTS

Compositional Analysis

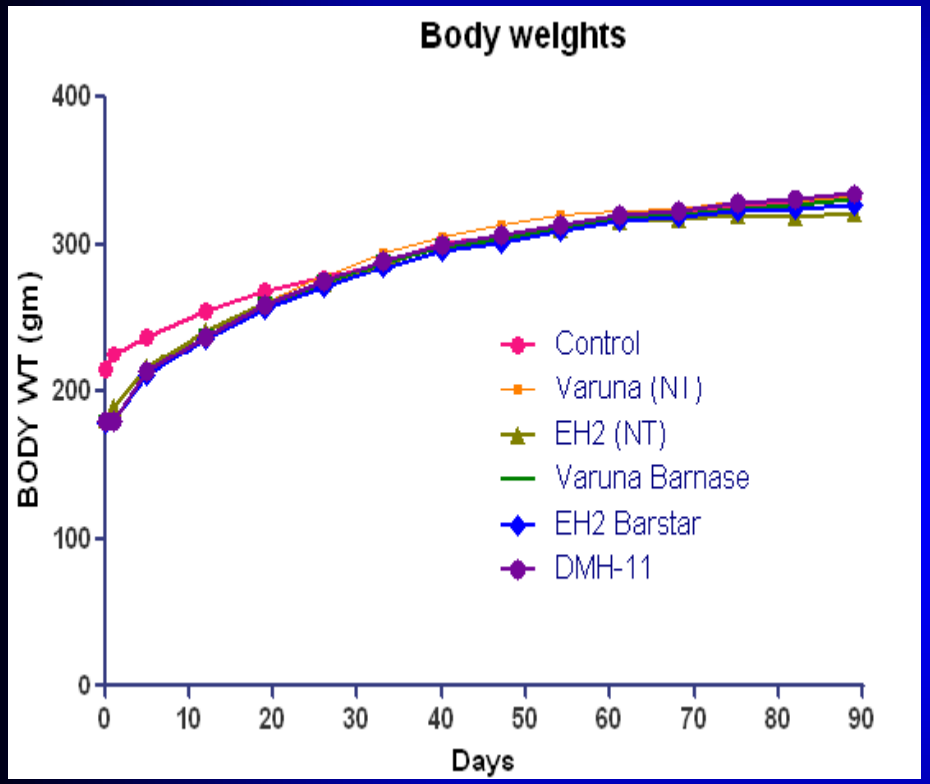
The compositional analysis:
Includes macro, micro nutrients were substantially equivalent inspite of the significant changes which may be due to agro-climatic changes.

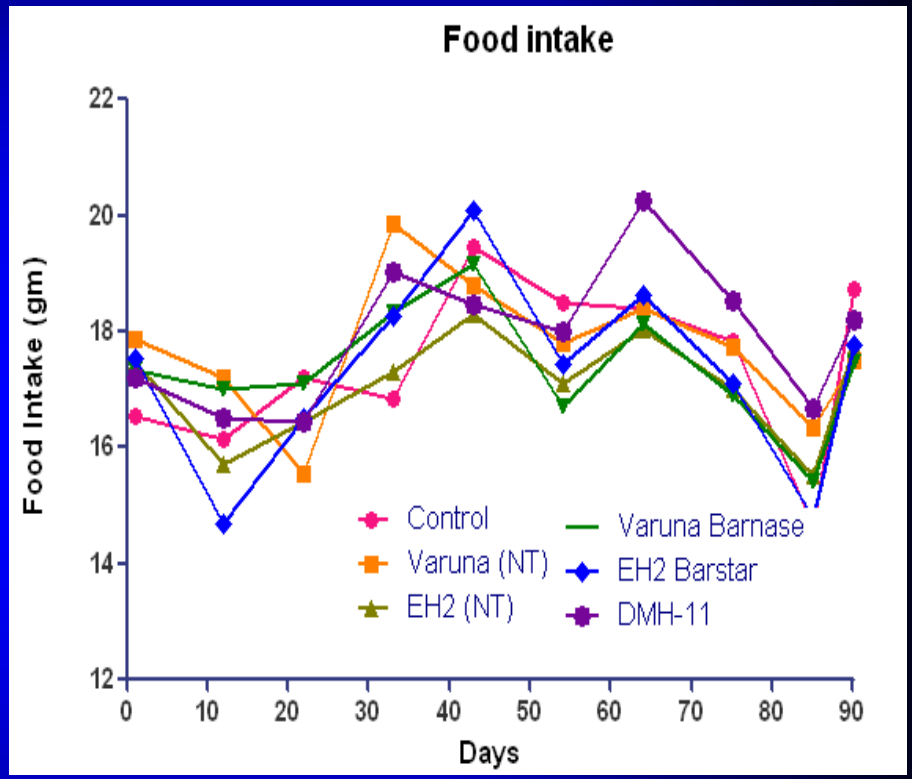
Acute Toxicity study (14days)

Recombinant Bar, Barstar and Barnase proteins

- ✓ No mortality in animals exposed to test protein.
- ✓ Gain in body weight, food intake was normal.

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



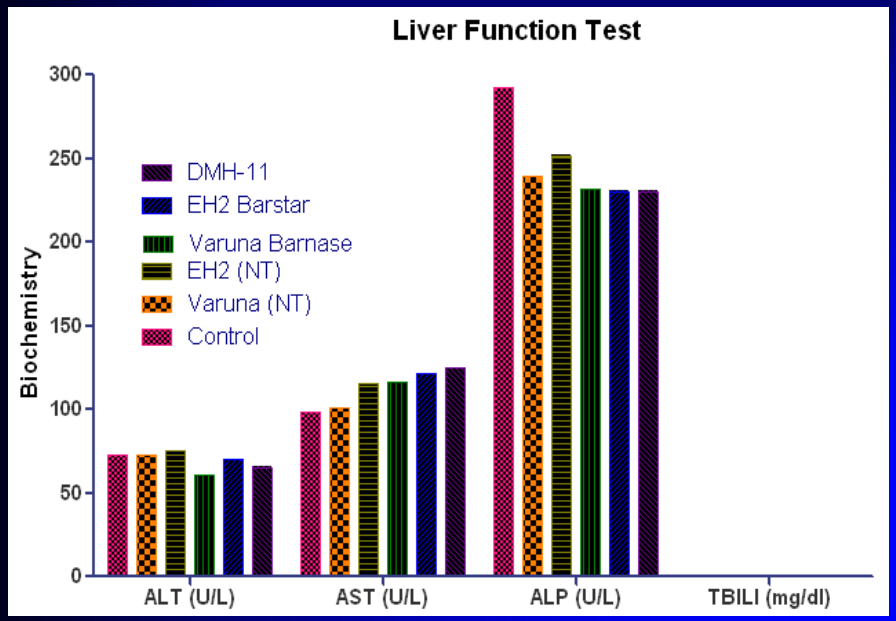


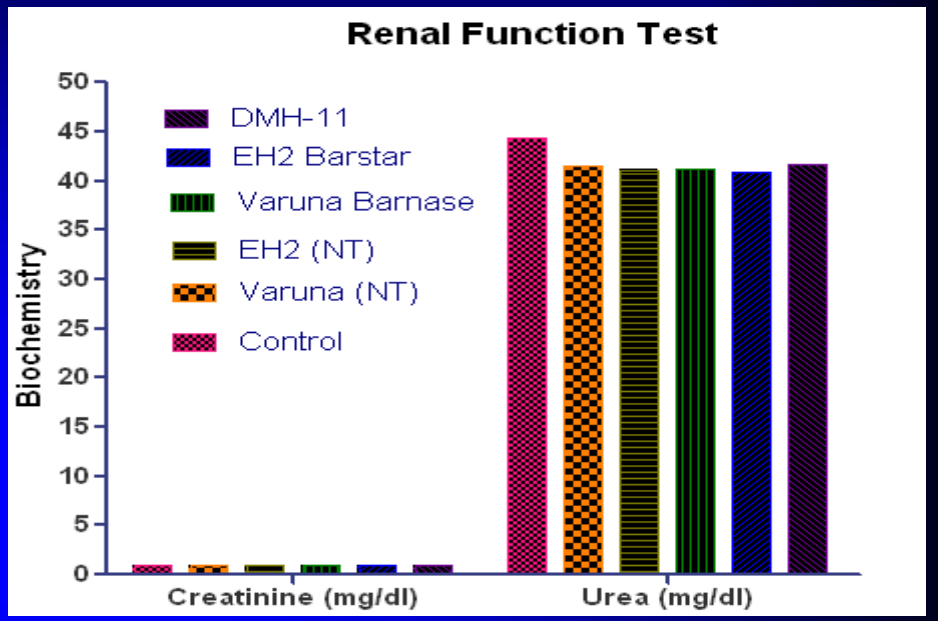
No significant difference in body weights, feed intake, and cage side activities.

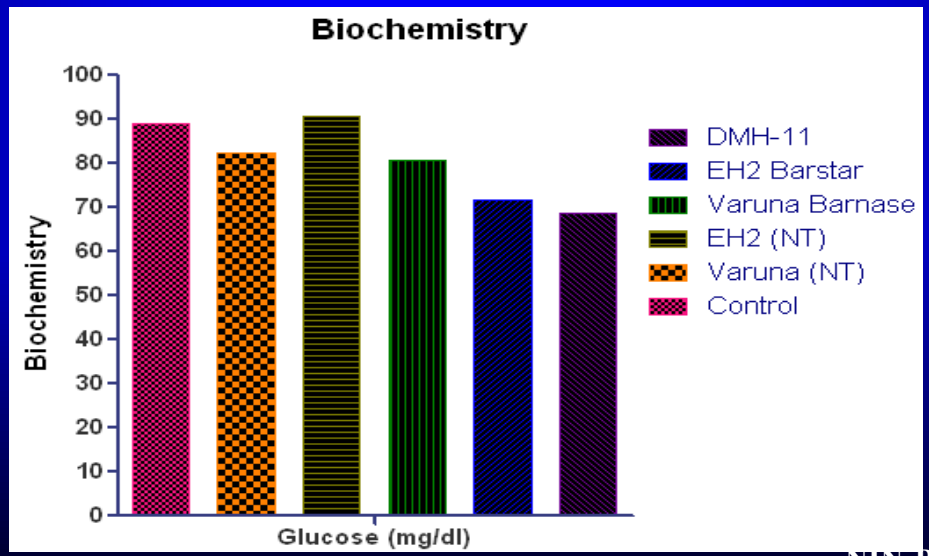
Values are expressed as Mean ± Standard Deviation **( ) No. of animals**

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

BIOCHEMICAL PARAMETERS – Sex Pooled (Rats)

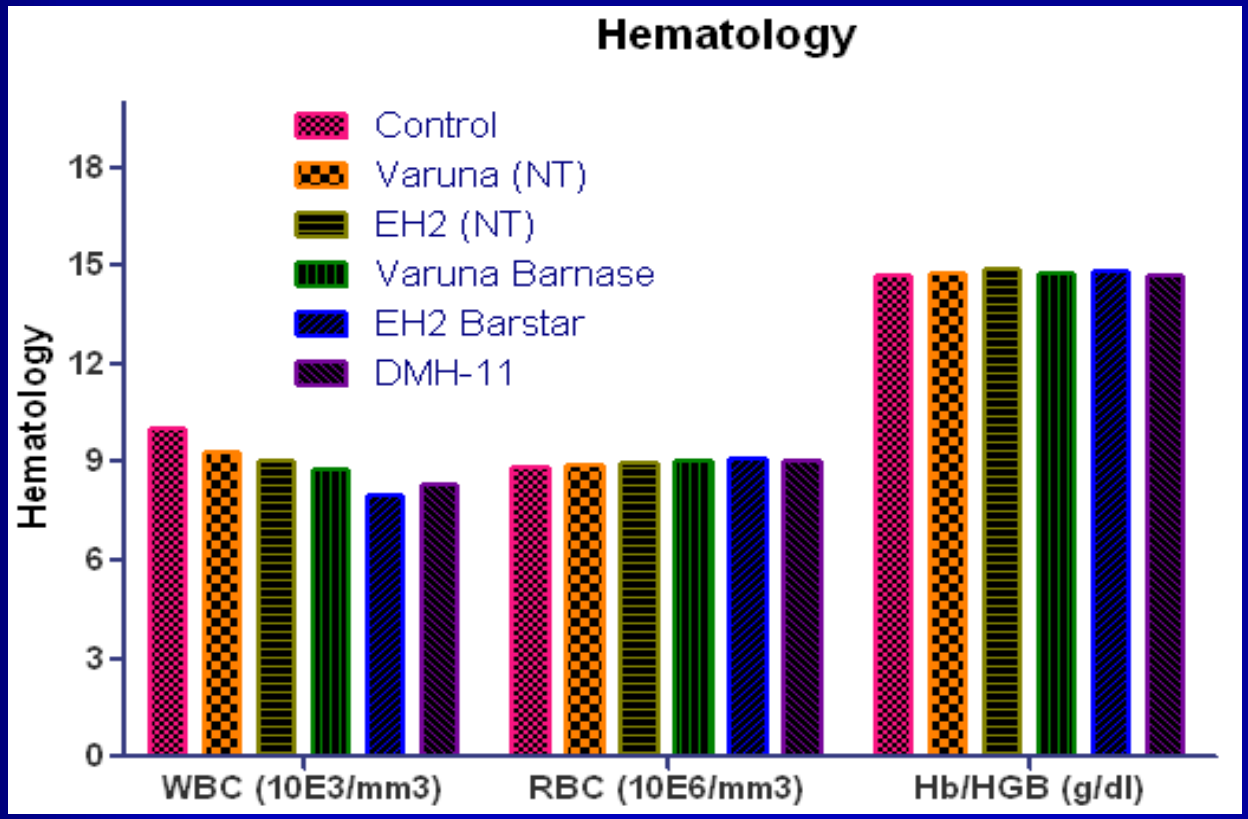






Values are expressed as Mean ± Standard Deviation ( ) No of animals

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



Hematology profile was within normal range.

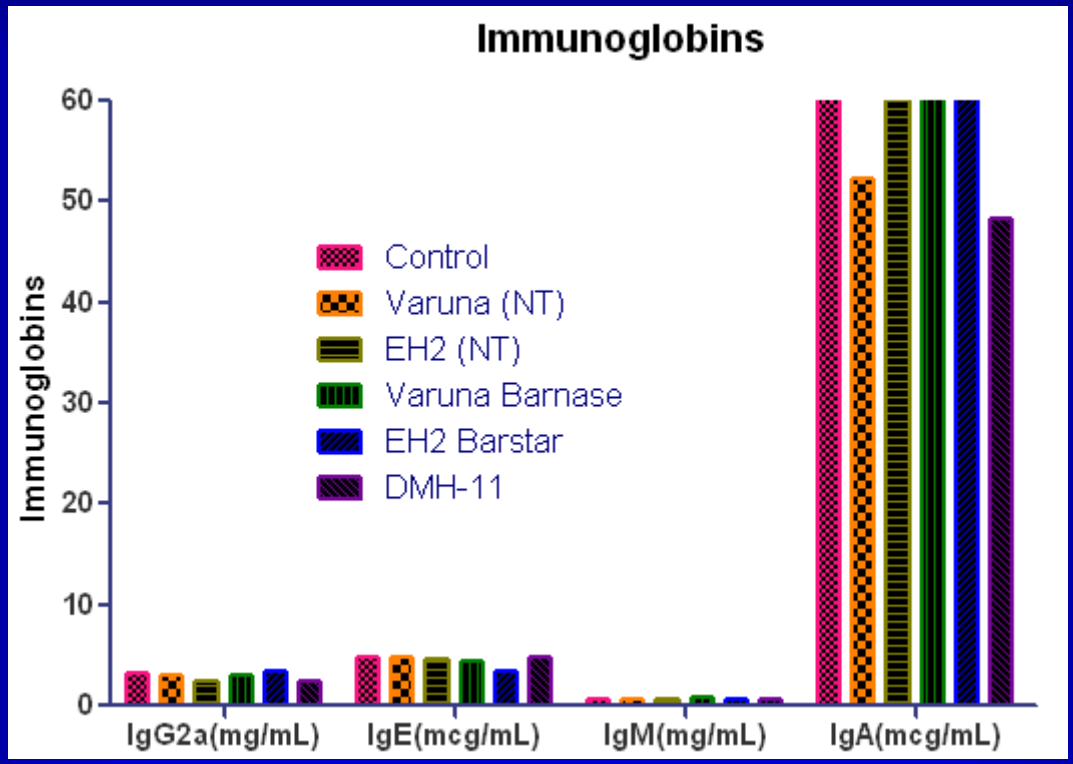
Values are expressed as Mean ± Standard Deviation ( ) No of animals

NIN-PCT

NIN-PCT

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

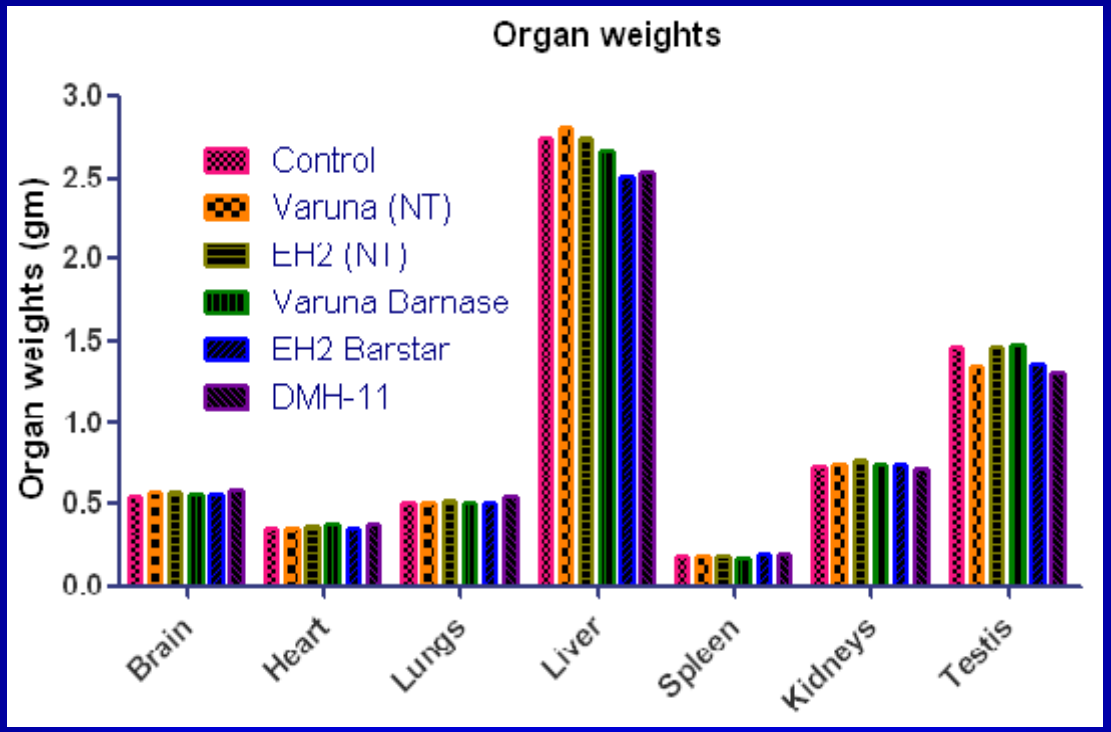
IMMUNOLOGICAL PARAMETERS - Sex Pooled (Rats)



- 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 see in the animals.

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

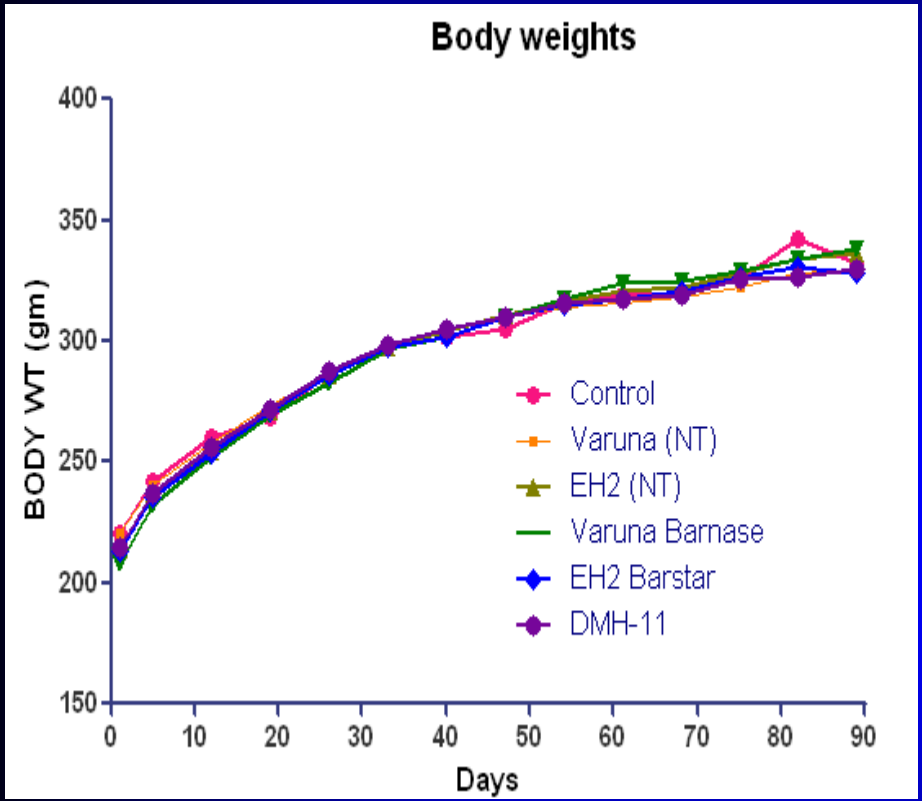
Sub chronic toxicity study of leaves from two events of Transgenic *B. juncea*
ORGAN WEIGHTS - Sex Pooled (Rats)

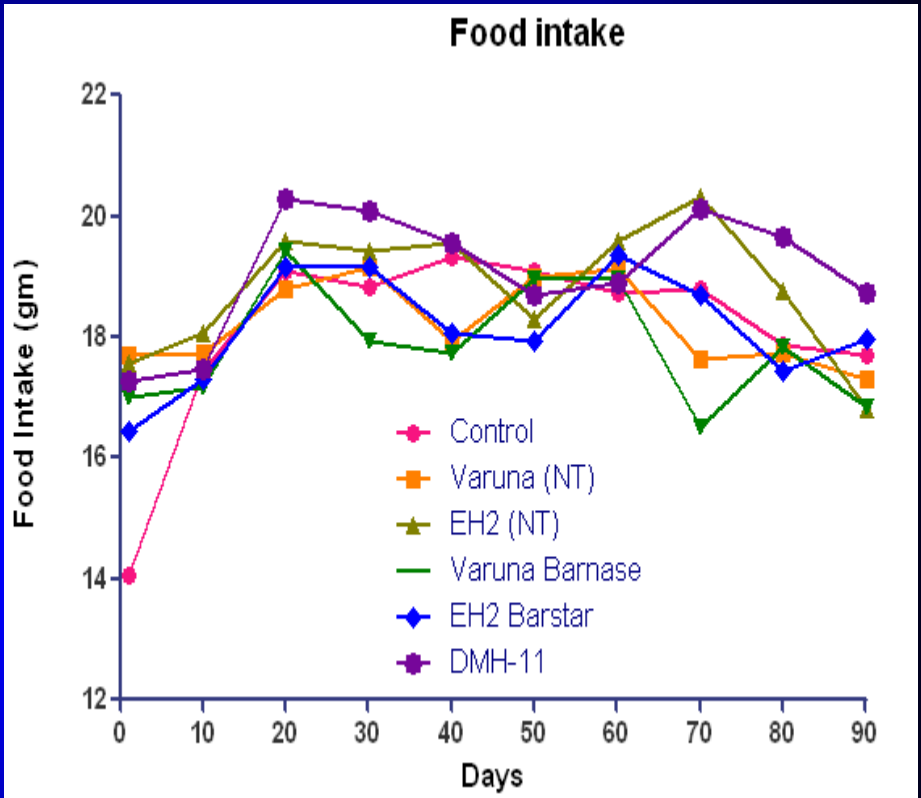


- No mortality in any group of animals which received transgenic or non–transgenic leaves of *B. juncea* at the dose of 0.4g/rat/day for 90 consecutive days.
- Histopathology evaluation was also unremarkable.

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

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





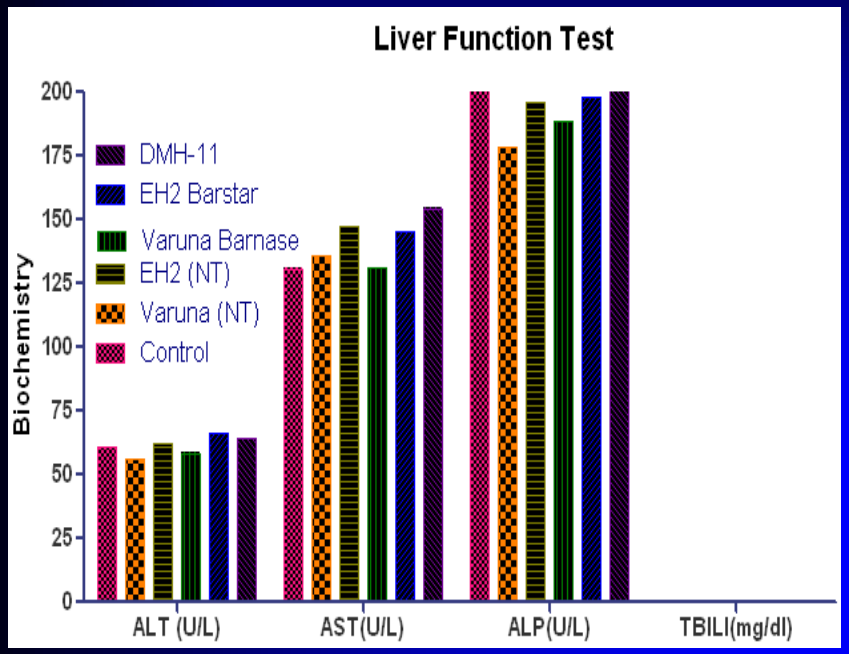
No abnormal findings with reference to gain in body weight, feed intake, cage side activity and clinical observations.

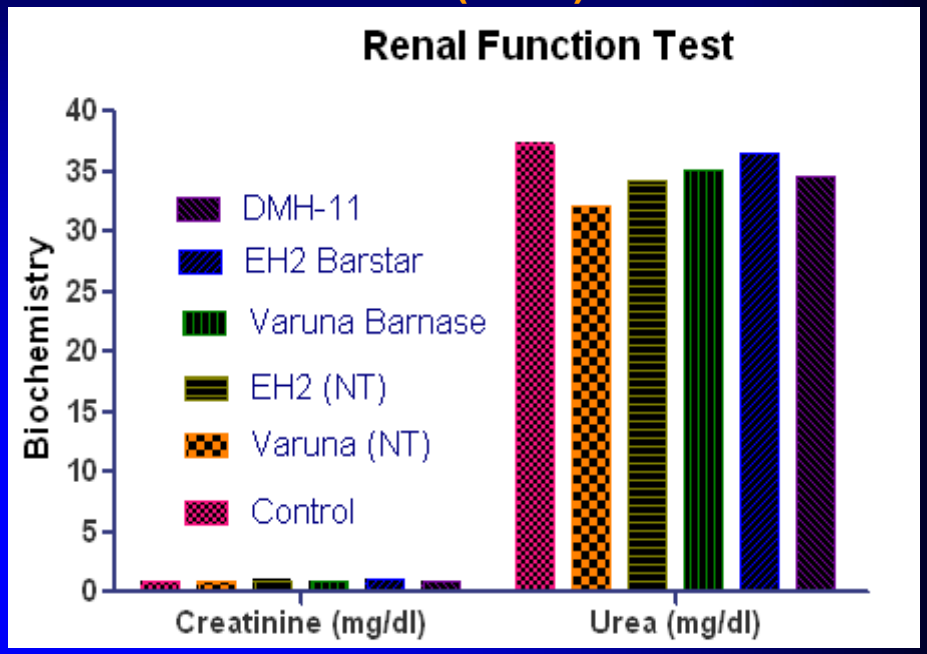
Values are expressed as Mean ± Standard Deviation ( ) No of animals

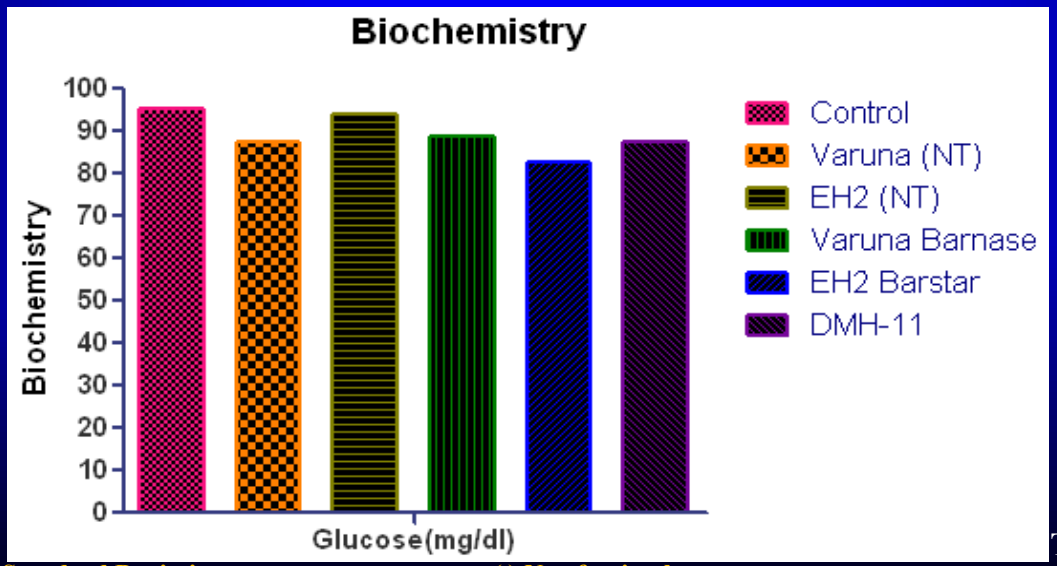
NIN-PCT

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

BIOCHEMICAL PARAMETERS - Sex Pooled (Rats)







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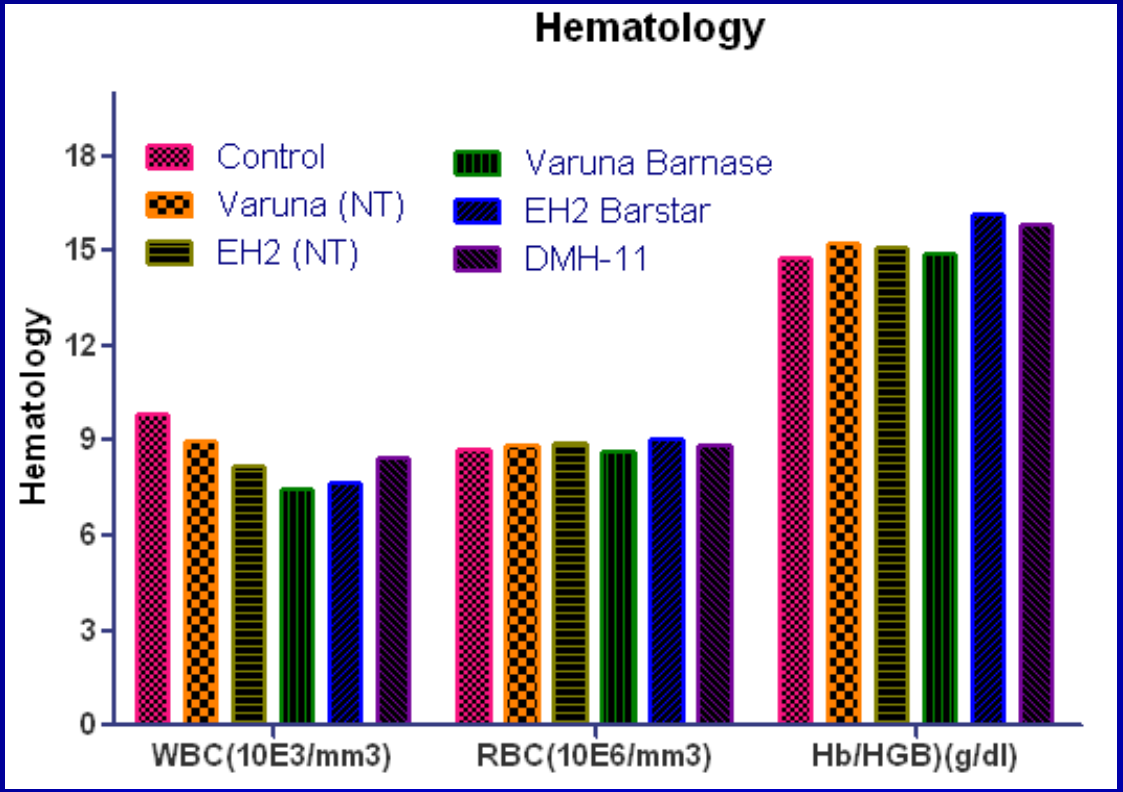
Values are expressed as Mean ± Standard Deviation ( ) No of animals

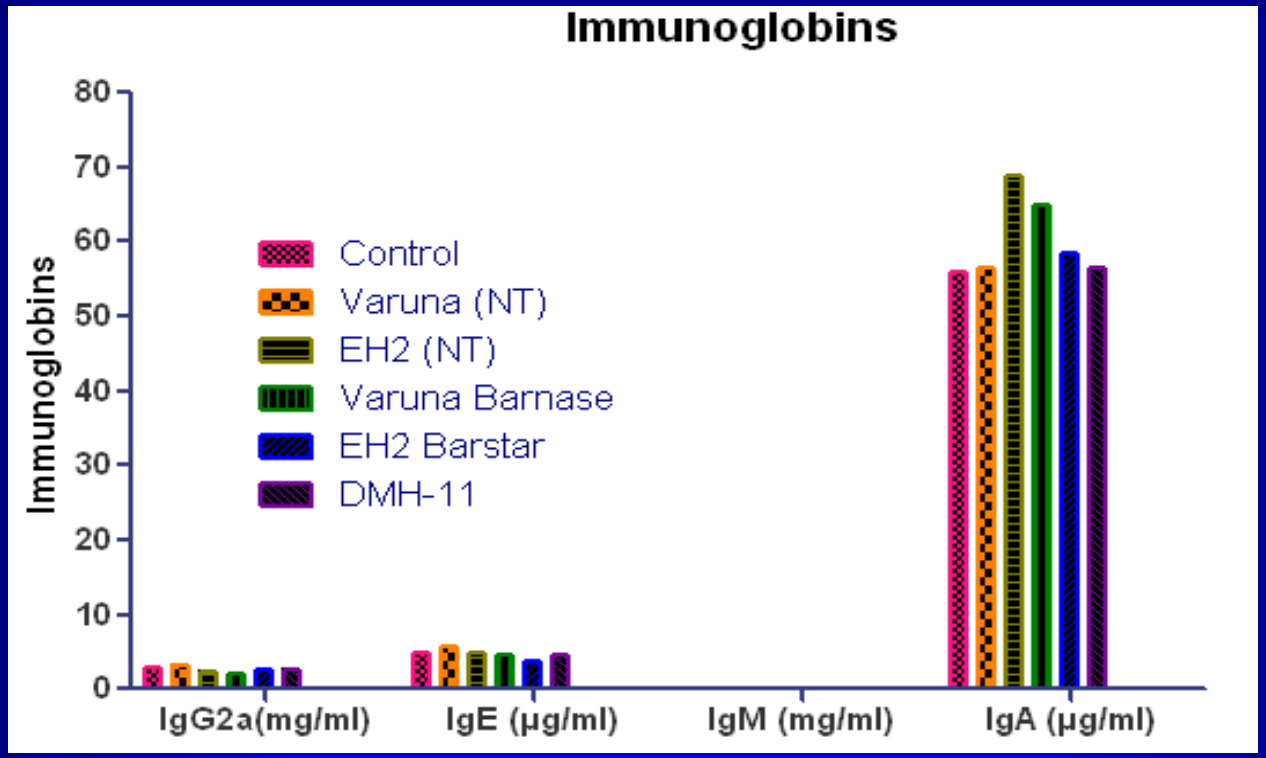
Sub chronic toxicity study of seeds from two events of Transgenic *B. juncea*
HEMATOLOGY - Sex Pooled (Rats)



Hematological observations were within normal range.

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

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

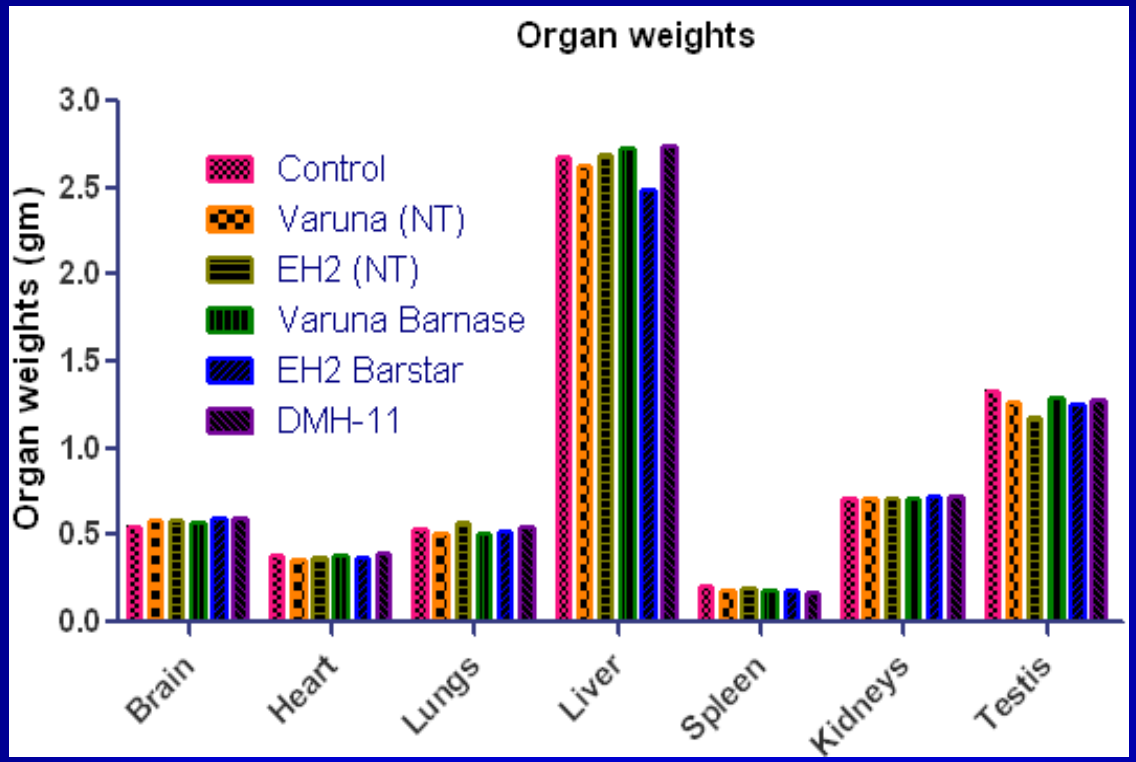


- Serum total IgG2a, IgE, IgM and IgA levels were not altered with transgenic and were compared with non–transgenic *B. juncea* seed fed animals.
- No allergy symptoms seen in the test and control animals.

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

NIN-PCT

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 ± Standard Deviation ( ) No of animals

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

HISTOPATHOLOGY - (Rats)

The histopathological result was

also seen to be unremarkable.

NIN-PCT

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 ≡ 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 ≡ 0.53gm human intake

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* **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**

? 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

Source: CPT, 2007, 82, 211-214

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	

CAN WE FACE CHALLENGES ?

Bt Brinjal is safe, claims NIN

However, Activists Against GM Crops Need To Be Convinced

TIMES NEWS NETWORK

READY TO COOK?

Hyderabad: Is Bt Brinjal safe? The demonisation of BT crops got a push with the parliamentary committee on agriculture in its report submitted last month commenting that transgenics in food crops would be fraught with unknown consequences. But the Hyderabad-based National Institute of Nutrition (NIN) of the Indian Council of Medical Research (ICMR) says that Bt Brinjal is safe.

A voluminous 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.

B Dinesh Kumar, deputy director, Food and Drug Toxicology Research Centre, at the NIN told TOI that in every respect, Bt Brinjal was found to be safe. "What now needs to be done is open field trials," Dinesh Kumar said.

However, the problem arises here. In order to analyse the effects of Bt Brinjal in human consumption, it first needs to be introduced in the market. The Genetic Engineering Approval Committee (GEAC) will then need to allow introducion of Bt Brinjal, at least in a limited way, so that the effects can be evaluated.

When Bt Brinjal was sought to be introduced in the market a few years ago, it led to a controversy. However, on February 9, 2010, the ministry of environment and forests imposed a moratorium on Bt Brinjal. In the absence of scientific consensus and opposition from state governments and others, the ministry decided to impose a moratorium on the commercialisation of Bt Brinjal until all concerns expressed by the public, NGOs, scientists and the state government were addressed adequately.

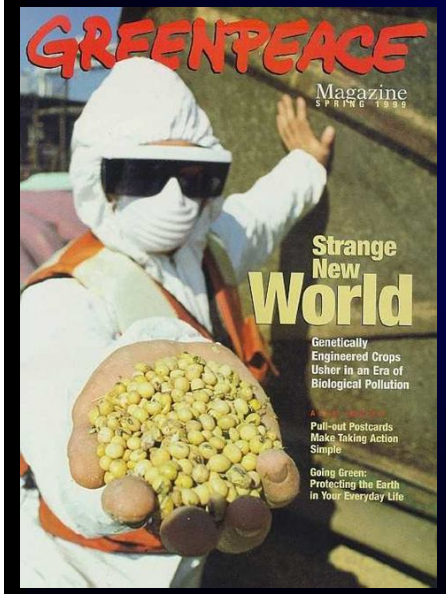
Among those actively opposed to the introduction of Bt Brinjal is P M Bhargava, founder-director of the Centre for Cellular and Molecular Biology, Hyderabad, who was nominated by the Supreme Court to the GEAC.

Bhargava who had even argued against Bt Cotton told the parliamentary committee on agriculture that GM organisms could be introduced only after adequate testing was done.

The pre-clinical tests conducted at the NIN show that Bt Brinjal is safe but activists who are against genetically modified crops need to be convinced about the study and its results.

YES !

LET US PREPARE IDENTIFY THE INTERVENTION STRATIGIES



FACTS AND EXPERIENCE

- 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

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RETRACTED

Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

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ABSTRACT

The health effects of a Roundup herbicide and a genetically modified maize (from 11% in the diet), cultivated with or without Roundup, and Roundup alone (from 0.5 ppb in water), were studied 2 years in rats. In females, all treated groups died 2–3 times more than controls, and more rapidly. This difference was visible in 3 male groups fed GMO. All results were hormone and sex dependent, and the pathological profiles were comparable. Females developed large mammary tumors almost always more often than and before controls, the pituitary was the second most disabled organ; the sex hormonal balance was modified by GMO and Roundup treatments. Treated males, liver congestions and necrosis were 2.5–5.5 times higher. This was confirmed by optic and transmission electron microscopy. Marked and severe kidney nephropathies were also generally 1.3–2.3 greater. Males presented 4 times more large palpable tumors than controls which occurred up to 600 days earlier. Biochemistry data confirmed very significant liver and kidney chronic deficiencies; for all treatments and both sexes, 783 of the altered parameters were kidney related. These results can be explained by the non linear endocrine-disrupting effects of Roundup, the metabolic pathway overexpression of the transgene in the GMO and its metabolic consequences.

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1. Introduction

There is an ongoing international debate as to the necessary length of mammalian toxicology studies in relation to the consumption of genetically modified (GM) plants including regular metabolic analyses (Seralini et al., 2011). Currently, no regulatory authority requires mandatory chronic animal feeding studies to be performed for pesticides and formulated pesticides. However, several studies consisting of 90 day rat feeding trials have been conducted by the biotech industry. These investigations mostly concern GM maize and maize that are rendered either herbicide tolerant (to Roundup (R) in 80% of cases), or engineered to produce a modified *Bt* toxin insecticide, or both. As a result these GM crops contain new pesticide residues for which new maximal residual levels (MRL) have been established in some countries.

If the petitioners conclude in general that there is no major change in genetically modified organism (GMO) subchronic toxicity studies (Domingo and Giné Bordonaba, 2011; Hammond et al., 2004, 2006a,b), significant disturbances have been found and may be interpreted differently (Séralini et al., 2009; Spiroux de Vendômois et al., 2010). Detailed analyses have revealed alterations in kidney and liver functions that may be the signs of early chronic diet intoxication, possibly explained at least in part by pesticide residues in the GM feed (Séralini et al., 2007; Spiroux de Vendômois et al., 2009). Indeed, it has been demonstrated that R concentrations in the range of 10^{-9} times below the MRL induced endocrine disturbances in human cells (Gasnier et al., 2009) and toxic effects thereafter (Benachour and Seralini, 2009), including *in vivo* (Romano et al., 2012). After several months of consumption of an R-tolerant soy, the liver and pancreas of mice were affected, as highlighted by disturbances in sub-nuclear structure (Malatesta et al., 2008a, 2002a,b). Furthermore, this toxic effect was reproduced by the application of R herbicide directly to hepatocytes in culture (Malatesta et al., 2008b).

Abbreviations: GM, genetically modified; R, Roundup; MRL, maximal residual levels; GMO, genetically modified organism; OECD, Organization for Economic Cooperation and Development; GT, glutamyl-transferase; PCA, principal component analysis; PLS, partial least-squares; OPLS, orthogonal partial least-squares; NIPALS, Nonlinear Iterative Partial Least Squares; OPLS-DA, Orthogonal Partial Least Squares Discriminant Analysis; G, glycogen; L, lipid droplet; N, nucleus; R, rough endoplasmic reticulum (on microscopy pictures only); U, urinary; Ure, excreted in urine during 24 h; APPT, Activated Partial Thromboplastin Time; MCV, Mean Corpuscular Volume; PT, Prothrombine Time; RBC, Red Blood Cells; ALT, alanine aminotransferase; MCHC, Mean Corpuscular Hemoglobin Concentration; A/G, Albumin/Globulin ratio; WBC, White Blood Cells; AST, aspartate aminotransferase.

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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

PUBLIC-PUBLIC & PRIVATE PARTNERSHIP PROGRAMS AT NIN

NATIONAL INSTITUTE OF NUTRITION (ICMR)

CENTRE FOR ADVANCED RESEARCH FOR PRE-CLINICAL TOXICOLOGY (ESTD. 1998)

Govt / Public Institutes

Drugs

- IISC, Bangalore
- IIL, Hyderabad
- AIIMS,New Delhi
- PGI, Chandigarh
- CCRAS, New Delhi
- DCGI, GoI
- CDRI, Luknow
- ANGRAU, Hyderabad
- DST

Foods

- NDDB, Anand
- ANGRAU, Hyderabad
- University of Delhi
- IICT, Hyderabad
- ICAR (NAIP)
- Tea Board of India

Private Organization

Drugs

- Shanta Biotech
- Dr. Reddy Labs
- Panacea
- Biological E lab
- Sipra Laboratory Pvt. Ltd.
- Virchow biotech Pvt. Ltd.
- Asian Herbex Pvt. Ltd.
- Cadila Pharma Pvt. Ltd.
- ISSAR Pharma. Pvt. Ltd.
- Stempeutics Pvt. Ltd.
- Vanjan Biopharma Pvt. Ltd.
- Zenotech Pvt. Ltd.
- Crystalin research Pvt. Ltd.
- Sudhershan biotech
- Clonz biotech
- Bioviz Pvt. Ltd.

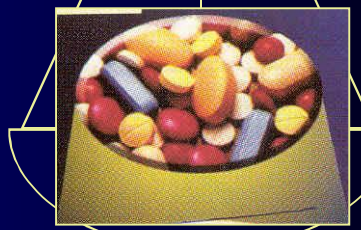
Foods

- Bejosheetal
- Mahyco Seeds
- JK Seeds
- Vasmo Foods
- Foods, Fats & Fertilizers Limited.
- Elan Pharma (India) Pvt. Ltd.

THE UNBEATABLES

THANK 'U'

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 &Kamla Krishnaswamy